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MATURATION OF McINTOSH APPLES IN RELATION TO STARCH LOSS AND ABSCISSION[1]

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[Received for publication July 7, 1958]

ABSTRACT

A field test, which utilizes a sequence of starch-iodine patterns for estimating picking time in McIntosh, was related to the starch content of that fruit. Further, it was observed that starch content and abscission appeared to be associated and that this association varied with the seasonal temperature.

INTRODUCTION

In Eastern Canada, where McIntosh is the principal apple variety, larger proportions of this fruit are being placed in controlled atmosphere storage every year. To minimize scald and to ensure that only the highest quality apples enter controlled atmosphere storage, the fruit must be left on the trees longer than is frequently practised. This entails risk of loss through drop and the display of overmaturity symptoms in storage. Hence timing of harvest has become more exacting than was necessary in the past, and more reliable techniques of assessing physiological age and rapidity of fruit development are required.

At Ottawa, some of the commonly accepted criteria of maturation were studied as recently as 1948 (5). These criteria included such factors as ground colour, blush, acidity, soluble solids, firmness, respiration, and peroxidase activity. The most satisfactory indicator of maturation however appeared to be the starch patterns as described by Davis and Blair (1). These starch-iodine patterns have been used successfully at Ottawa for many years but have not found general acceptance. In this report the starch-iodine pattern technique (1) is described and certain aspects of its application are examined.

A more widely used indicator of maturation is the so-called "grower index" which is simply the ease with which the fruit separates from the spur. As the fruit matures the strength of attachment on the tree weakens and eventually it drops, at which time the fruit may be described as mature. Prior to abscission the amount of immaturity may be measured in days, that is, the lapse of time required to produce natural drop.

This paper is a report of experiments conducted over a 5-year period, 1952-56, which were designed to examine the timing of abscission in relation to starch disappearance during the maturation of McIntosh apples.

[1]Joint contribution from Horticulture Division, Experimental Farms Service (Contribution No. 937), and Chemistry Division, Science Service (Contribution No. 403), Canada Department of Agriculture, Ottawa, Ont.

METHODS AND MATERIALS

During 1952-1956 three sets of data were collected annually from the crops of a single McIntosh tree which had uniform cultural treatment and no pre-harvest sprays. The date of "mean maturity" was determined from data based on the natural drop of apples from the tree. Changes in starch-iodine pattern (1) or "index" were observed at frequent intervals during the harvest season and the starch content of the same apples was estimated chemically.

The date of mean maturity was determined as follows:—

Some time in advance of active dropping, a clean-up was made of the fallen fruit and these were discarded. Each day thereafter the windfalls were collected and weighed and at the end of the season the weights were converted to percentages of the total dropped crop. The date of mean maturity was then calculated by the following formula:

$$D = [(W_1 \times N_1) + (W_2 \times N_2) + (W_3 \times N_3) \text{——} (Wn \times Nn)] \div 100$$

Where D = day of mean maturity

W_1——n = weight of collections (1——n) expressed as a percentage of total dropped crop

N_1——n = time in days for collections (1——n) following the initial clean-up.

This relation for finding the date of mean maturity or a middle condition of abscission was chosen for its simplicity and adequate accuracy. Generally 80 per cent of the fruit drop was accounted for in the linear portion of the drop accumulation curve, and though the periods of collection ranged from 40-70 days, over 60 per cent of the drop was accounted for in all cases within a 15-day period. The heavy centralized drop meant that small fractional collections on the extremities bore little influence on the final calculation.

Sampling for indexing and starch measurement was started usually in the latter part of August. Random samples of ten apples each were removed from the tree at 9 a.m. at intervals of 7 days or less. Each apple was cut in half at right-angles to the principal axis. Five calyx ends and five stem ends were immediately indexed by immersing the cut faces in potassium iodide-iodine solution and comparing the resulting patterns with the maturity chart illustrated in Figure 1. When making comparison with the maturity chart emphasis was placed on the location (pattern) of the retreating starch granules rather than on the intensity of the blue-black coloration.

The opposite halves of the same ten apples were used for the chemical estimation of starch. The chopped tissue was mixed for 5 minutes in a Waring blender with an equal quantity of water. An aliquot of the suspension was treated with perchloric acid to dissolve the starch and, after a short period of digestion, the acid was neutralized, potassium iodide-iodine solution was added and the resultant blue colour was measured on a colorimeter. The readings were referred to a standard chart prepared from pure potato starch which was shown to have approximately the same amylose-amylopectin ratio as McIntosh apple starch. The amylose

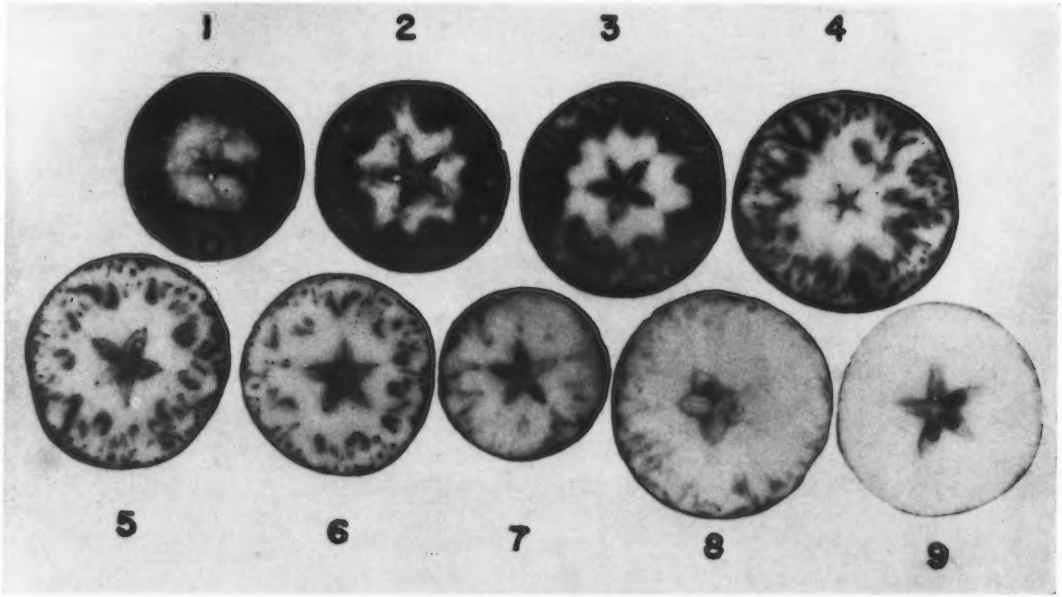
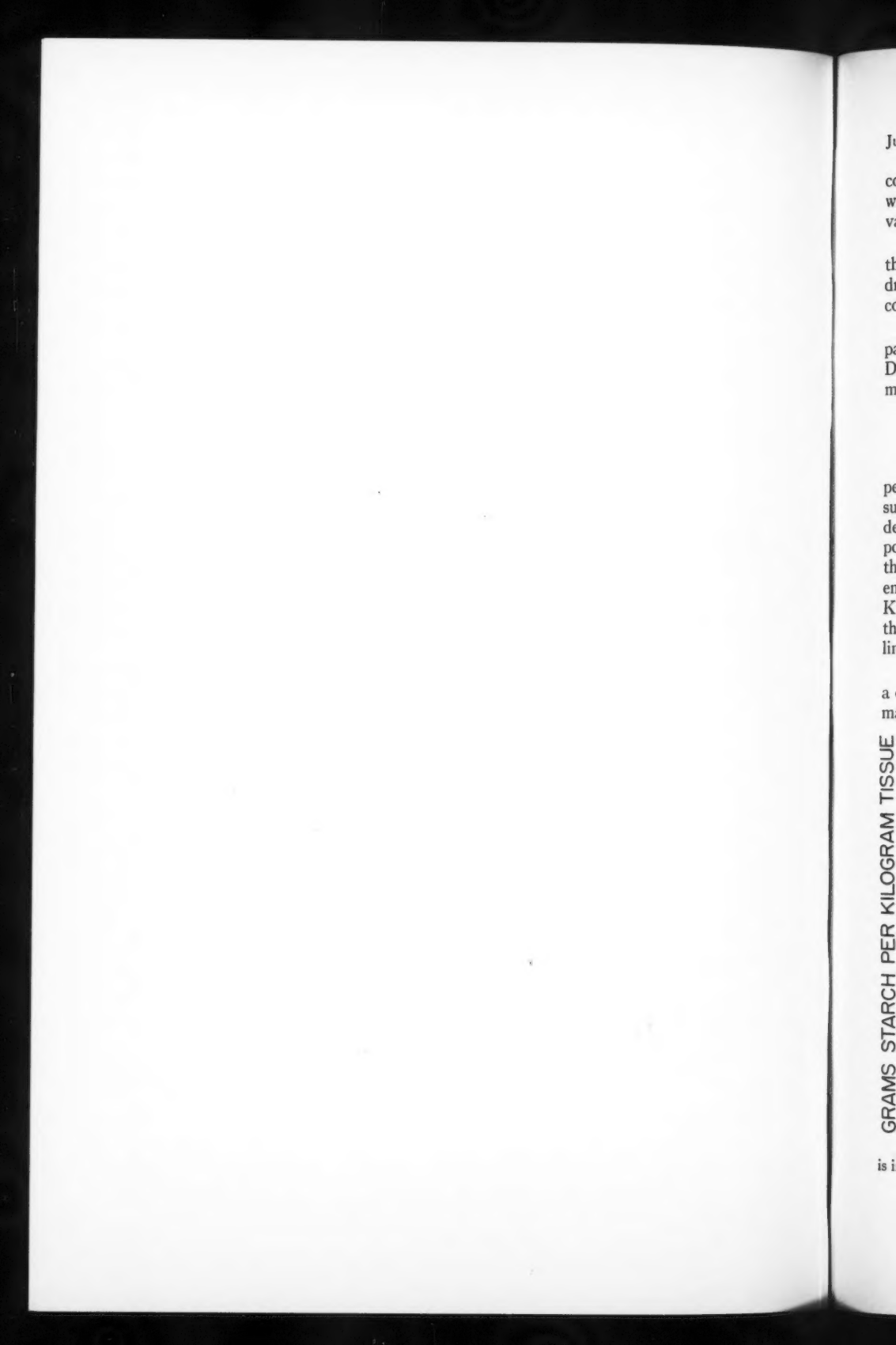


FIGURE 1. Maturity chart of Davis and Blair.


GRAMS STARCH PER KILOGRAM TISSUE

content of starch from McIntosh apples was found to be 27.1 per cent, which was in reasonable agreement with the values for starch of other varieties listed by Carter and Neubert (2).

In addition to the starch measurements made on the attached fruit, the indexing test was applied to the windfalls whenever ten or more apples dropped within a 24-hour period. These and the other starch tests were computed into simple averages.

Temperature data were available in "Weather Summary and Comparative Data", issued by the Central Experimental Farm, Ottawa. Daily mean temperatures were computed by averaging the maximum and minimum readings recorded in this publication.

RESULTS AND DISCUSSION

The pre-harvest decline in starch was generally measured over a period of 5 to 6 weeks. In Figure 2 the results of this analysis on five successive crops are presented in graphical form. It was found that starch decline progressed in a linear manner and straight lines were fitted to the points on the graph by the method of least squares. In later discussion the straight lines are extended to the horizontal axis for zero starch reference, recognizing that in the limit the declines are possibly asymptotic. Krotkov and Helson (3) measured starch changes in McIntosh apples throughout the complete period of growth. Their results also show a linear decline during the latter stages of growth.

Index readings were also treated in a linear manner and where applicable a curve was fitted by the method of least squares. When comparison was made on rectangular plot it was found that index readings were directly

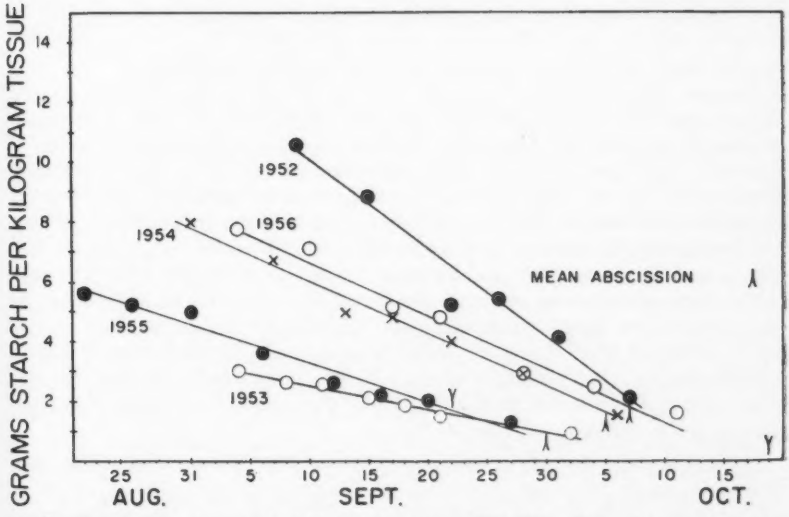


FIGURE 2. Starch content of McIntosh apples, 1952-1956. Date of mean abscission is indicated.

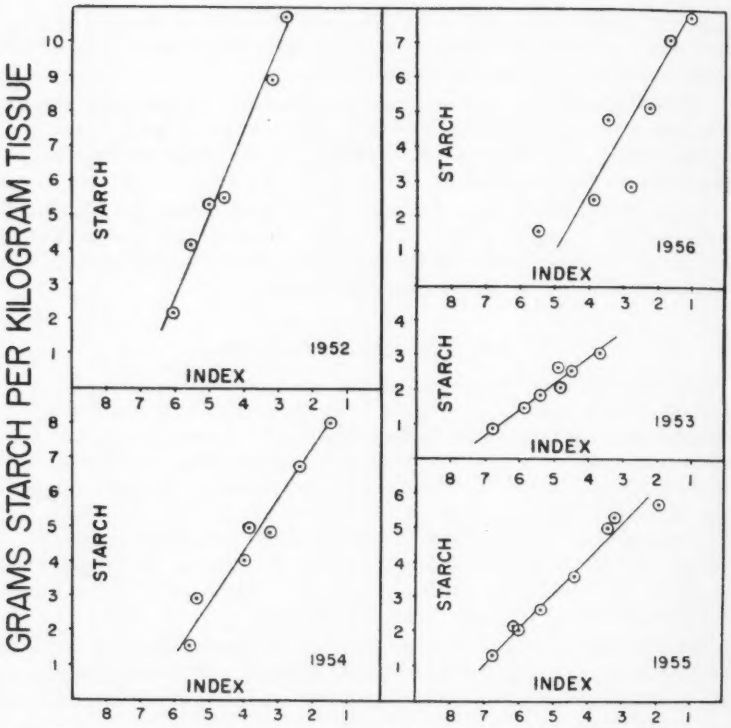


FIGURE 3. Index measurements compared with starch analyses, 1952-1956.

proportional to starch analyses for any one season (Figure 3). This proportionality, however, did not extend to comparisons between the seasons and it is noted that index 1 varies approximately from 5-14 grams of starch per kilogram of tissue. Apparently the maturity chart (Figure 1) functions as a kind of starch proportioning system whose proportionality factor varies from season to season. Further examination of Figure 3 revealed an inherent error in the design of the maturity chart in that extrapolated data fail to reach index 9 and this bias also varies from year to year. The reason for this difficulty is embodied in the chart, in that early changes represent major losses in starch content and later changes represent minor losses. Examination of original data substantiated this latter cause of bias, a matter which would easily be corrected in future redesigning of the maturity chart. Most index readings used in this paper were associated with the middle of the maturity chart and did not appear to be seriously affected with bias.

First indications of a relation between starch and abscission were observed in the indexing of windfalls. Harvest drop tended to occur at a fixed index or a fixed concentration of starch for a season. There were variations, of course, resulting from wind, impact of falling apples, etc., but

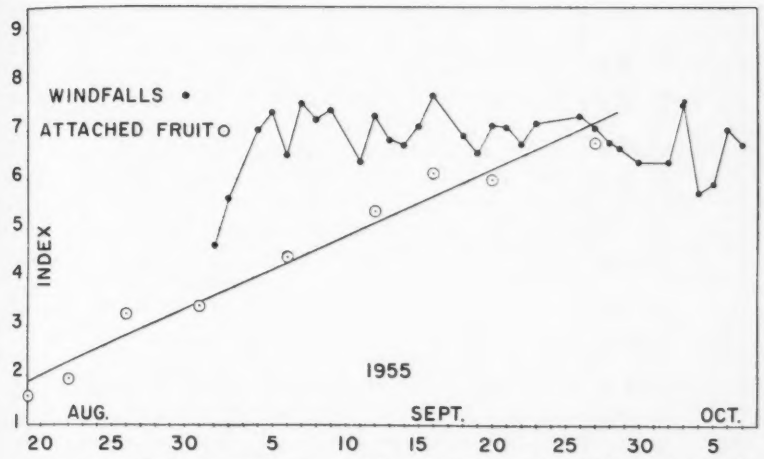


FIGURE 4. Index measurements on McIntosh windfalls and attached fruit in 1955.

within reasonable limits there appeared to be a fixed seasonal concentration of starch at which the fruit dropped. Typifying this condition are the 1955 data shown in Figure 4. Over all five seasons the mean index of windfalls varied between 7.0 and 8.5. During the same 5-year intervals the index of the attached fruit at mean maturity varied between 5.7 (1954) and 7.2 (1956). The low 1954 index resulted at least in part from the influence of winds of hurricane force ("Hurricane Hazel") which abruptly removed the last 12 per cent of the fruit. The range of starch concentrations in the attached fruit at mean maturity for the 5 years varied between 0 and 2.16 grams per kilogram of tissue, or possibly up to a maximum of one-fifth of the original starch concentration.

It would have been ideal and convenient had the time of abscission coincided with complete loss of starch. However, such a condition rarely occurs and generally abscission precedes the complete loss of starch in McIntosh. This lead, or occasionally a lag, can be measured in days by extrapolating the starch lines in Figure 2 to zero or to some other fixed reference. It has been observed that the tendency to remain attached or to drop is heavily dependent on the prevailing seasonal temperature (4). Data revealed very little, if any, influence of prevailing temperature on starch decline in either comparisons within a season or comparisons between seasons, though undoubtedly there must be an effect. It appears that the abscission lead or lag is a function of temperature. The time rates of starch degradation and of the processes causing abscission are involved and the latter are most affected.

If it is assumed that the lead or lag of abscission over the complete degradation of starch is directly influenced by the prevailing temperature, the question arises as to what period to consider for temperature computation. In this connection it was perhaps fortunate that the question of temperature boundaries was not immediately pressing. Having set the

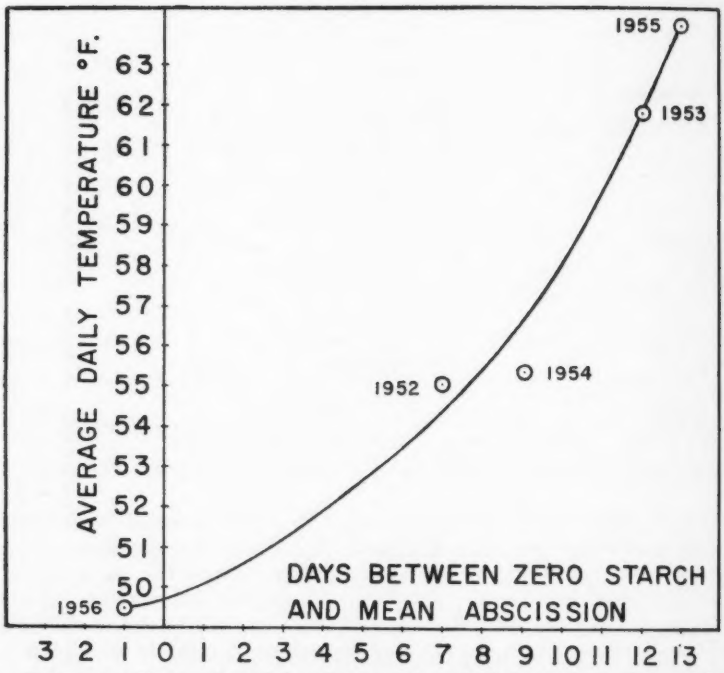


FIGURE 5. The number of days between mean abscission and complete starch disappearance in attached fruit related to seasonal temperatures.

latter limit arbitrarily at the calendar date of 90 per cent drop, the placement of the initial boundary made little difference whether considered as the first day of September or as the blossom dates. Figure 5 shows the number of days included between an extrapolated condition of zero starch and mean abscission referred to the average prevailing temperature. In Figure 5 the initial temperature boundary was considered to be the day when index 1 occurred, and was arrived at by the backward extrapolation of the index values; it seemed more logical to associate the initial tempera-

TABLE 1.—DATA USED TO DEMONSTRATE A TIME ASSOCIATION BETWEEN STARCH LOSS AND ABSCISSION

Year	Estimated initiation date of starch degradation	Mean abscission date	Calculated starch zero date	Date of 90% drop	Av. temp. deg. F.
1952	Aug. 23	Oct. 7	Oct. 14	Oct. 21	55.1
1953	Aug. 8	Sept. 30	Oct. 12	Oct. 7	61.8
1954	Aug. 25	Oct. 5	Oct. 14	Oct. 17	55.4
1955	Aug. 12	Sept. 22	Oct. 5	Oct. 1	64.0
1956	Sept. 8	Oct. 19	Oct. 18	Oct. 29	49.5

ture boundary more closely with the maturation processes being measured. Flexibility existed in the selection of the latter boundary as well, but it did seem advisable to terminate in advance of 100 per cent drop and so avoid a disproportionate elongation of the temperature computation period by a small quantity of non-typical fruit.

Table 1 lists the basic data for the Figure 5 plot. It is interesting to note the similarities in the years 1952 and 1954. When the upper asymptote was sketched in on a drop accumulation plot for the year 1954 ("Hurricane Hazel") and the mean maturity data recalculated it was found that the high winds accomplished an advance of at least 1.6 days. If such a correction were applied it would bring the 1954 data in line with other data in Figure 5.

In conclusion, then, there appears to be an association between the timing of the processes of abscission and the decline of starch in the McIntosh apple at harvest time. It further appears that this association is markedly influenced by the prevailing seasonal temperature. Appreciation of these two points should permit more accurate assessment of the degree of immaturity of the McIntosh fruit, and also should increase the acceptance of starch measurement as a harvest time guide.

ACKNOWLEDGEMENTS

The authors wish to thank F. B. Johnston for suggesting the starch analytical procedure used and A. B. Durkee who performed many of the analyses.

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RATES OF SEEDING IRRIGATED PASTURES[1]

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ABSTRACT

An experiment to determine the effects of different rates of seeding on stand establishment and early yield of an irrigated pasture mixture was conducted from 1953 to 1956. The seed mixture used, in parts by weight, was: smooth bromegrass 7, orchard grass 7, creeping red fescue 4; and white Dutch clover 2. Plots 6 ft. by 30 ft. were seeded at rates of 5, 10, 15, 20, 25, 30, 35, and 40 lbs. per acre, with a tractor-drawn, multiple V-belt seeder with double disk furrow openers spaced 7 in. apart. Herbage yields were determined by mowing a 3-ft. strip down the middle of each plot.

Good stands were obtained from all rates of seeding, but there were differences in rate of establishment, early yield, and botanical composition of the swards. The heavier rates developed stands more quickly, yielded more in the year of seeding, and were less subject to weed encroachment than the lighter rates. Orchard grass dominated under the heavier seeding rates, resulting in an undesirable balance of species. Although satisfactory stands were obtained using light rates, moderate ones were considered more economical because they developed a sward more quickly and produced higher yields in the year of seeding.

INTRODUCTION

In establishing an irrigated pasture, the cost of seed can be a major economic consideration. Farmers and extension men sometimes suggest that recommended rates of seeding are extravagant. They point out that, while one or two plants per square foot of soil will produce maximum or near maximum yields, seeding rates are designed to place up to several hundred seeds per square foot. However, if a heavier rate of seeding than the minimum requirement will ensure a stand there may be justification for using it, particularly under irrigation where one cannot afford to let the land lie idle. The question to be resolved is the distinction between adequate insurance and extravagance.

The experiments described herein were undertaken to gain information on the effects of a wide range of seeding rates on the establishment and early development of the irrigated pasture mixture recommended for southern Alberta. This study is not concerned with subsequent yield and botanical composition as these are profoundly affected by ensuing management practices. The work was done at the Experimental Farm, Lethbridge, from 1953 to 1956.

REVIEW OF LITERATURE

In 1949, an irrigated pasture mixture recommended for southern Alberta (1) consisted of: bromegrass 6; Kentucky bluegrass 2; orchard grass 2; alfalfa 2; and white Dutch clover ½ pound per acre. Calculating from tables given by Wheeler (7), this is a total of 160 seeds per square foot. At that same time a mixture being recommended in Montana (3)

[1] Contribution from the Forage Crops Section, Canada Agriculture Research Station, Lethbridge, Alta.
[2] Agrostologist and Head of Section, respectively.

consisted of bromegrass 3 to 4; Kentucky bluegrass 4 to 6; Alta fescue 3 to 4; orchard grass 4 to 6, and ladino clover 1 to 2 pounds per acre, or a total 252 to 464 seeds per square foot. Jones and Brown (4) listed several mixtures suitable for California, with seeding rates varying from 11 to 25½ pounds per acre. They pointed out that the standard mixture, when seeded at a rate of 14 pounds per acre, would place 135 seeds on each square foot of soil. They considered that a good stand would be obtained if less than 10 per cent of those seeds developed into strong plants, but that a liberal allowance should be made for wastage and loss.

Brougham (2) sowed various combinations of perennial and short-rotation ryegrass with red and white clover, at rates varying from 22 to 47 pounds per acre. The heavier rates yielded more than the lighter ones for 5 to 7 months after seeding, but thereafter there were no differences in yield. The lighter rates permitted more clover to develop in the mixture, and also more unsown species. Brougham observed that unsown species could be suppressed by increasing the seeding rate of the mixture, but since that also suppressed the clover he considered pre-seeding cultivation preferable. Parry (5) reported similar results from seeding a mixture of 2 strains of perennial ryegrass and white clover, at rates of 10, 13, 18, and 25 pounds per acre. The seeding was done in spring with a grain companion crop. Differences in stand due to rates of seeding were easily discernible following the grain harvest, but no differences in yield could be detected when the plots were harvested for hay in July of the following year. Plots seeded at the lighter rates had more clover and more bare ground than those seeded at the heavier rates until the end of the second year. Parry concluded that it would be more economical to use the lighter rates of seeding the grasses, and speculated that weed encroachment might be checked by increasing the proportion of clover in the mixture.

MATERIALS AND METHODS

The species used in this experiment were: smooth bromegrass (*Bromus inermis* Leyss.), orchard grass (*Dactylis glomerata* L.), creeping red fescue (*Festuca rubra* L.), and white Dutch clover (*Trifolium repens* L.). No. 1 commercial seed of all species was used.

Seedings were made in May of each year from 1953 to 1956. Prior to seeding, the land was thoroughly cultivated, harrowed and packed. A tractor-drawn, multiple V-belt seeder, with double-disk furrow openers spaced 7 inches apart, was used. Seed was placed at a depth of approximately one-half inch and the land packed again after seeding. Plots were 6 feet by 30 feet, and replicated four times in a randomized, complete block design. Sprinkler irrigation was used to ensure good moisture conditions for germination and subsequent growth.

The seed mixture used for all seedings was, in parts by weight: smooth bromegrass 7; orchard grass 7; creeping red fescue 4; and white Dutch clover 2. Seeding rates in 1953 were 5, 10, 15, 20, 25, 30, and 35 pounds per acre. In the years 1954 to 1956, a 40-pound rate was included also.

In 1953, when the tallest plants had reached a height of 4 inches, approximately 6 weeks after seeding, counts were made of the number of

seedlings present in 1 foot of row, at four random locations in each plot. The number of seeds sown per foot was known, through actual counts, so the percentage that established as plants could be calculated.

The growth on all plots was harvested twice in the year of seeding, and three to four times in the year following seeding. The method of harvesting was to cut a swath 3 feet by 25 feet down the middle of each plot, after the ends had been trimmed off to eliminate border effect. The herbage was weighed in the field to the nearest tenth of a pound, and a sample of approximately 600 grams was taken to the laboratory for dry matter determination. Botanical composition of the herbage, with respect to grass, clover, and weeds, was determined at the time of harvest, by hand separation of a sample from each plot.

RESULTS

The relationship between the number of seeds sown and the number of plants established, in 1953, is shown in Table 1. With all species, increasing the seeding rate resulted in a greater stand density at the time of counting. The species showed differences in percentage establishment, brome and clover being lower in general than orchard grass and fescue.

TABLE 1.—EFFECTS OF SEVEN SEEDING RATES ON PLANT ESTABLISHMENT 6 WEEKS AFTER SEEDING IN 1953

Rate of seeding per acre (lb.)		Brome	Orchard	Fescue	Clover	Total
5	Seeds sown/ft.	4	10	6	4	24
	Seedlings established/ft.	1	4	5	3	13
	% establishment	25	40	83	75	54
10	Seeds sown/ft.	7	19	11	9	46
	Seedlings established/ft.	2	13	6	3	24
	% establishment	28	68	54	33	52
15	Seeds sown/ft.	10	29	16	13	68
	Seedlings established/ft.	3	10	10	5	28
	% establishment	30	34	62	38	41
20	Seeds sown/ft.	14	39	22	17	92
	Seedlings established/ft.	5	15	11	5	36
	% establishment	36	38	50	29	39
25	Seeds sown/ft.	18	49	28	22	117
	Seedlings established/ft.	4	20	12	6	42
	% establishment	22	41	43	27	36
30	Seeds sown/ft.	21	59	33	26	139
	Seedlings established/ft.	5	24	16	6	51
	% establishment	24	41	48	23	37
35	Seeds sown/ft.	24	69	38	30	161
	Seedlings established/ft.	5	33	22	7	67
	% establishment	21	48	58	23	42

20 lb. 10 lb. 5 lb. 35 lb.

FIGURE 1. A view of some of the plots as they appeared 8 weeks after seeding in 1953.

TABLE 2.—PERCENTAGE BOTANICAL COMPOSITION* 12 WEEKS AFTER SEEDING A GRASS-CLOVER MIXTURE AT SEVEN DIFFERENT RATES IN 1953

Rate of seeding per acre (lb.)	Grass	Clover	Weeds
5	54	20	26
10	78	16	6
15	80	10	10
20	86	5	10
25	94	4	2
30	92	5	2
35	95	3	2

* Based on oven-dry weights

Also, after an initial sharp decline, there was a slight tendency for the percentage establishment of clover to decrease as seeding rates increased. This tendency was not apparent with the grasses.

Subsequent to the counting of the seedlings there appeared to be a reduction in plant population, particularly in plots seeded at the lighter rates. A second attempt was made to count the numbers of plants, but some had grown so large that it was impossible to distinguish individuals. The appearance of some of the plots 8 weeks after seeding is shown in Figure 1.

On August 10, at the time of the first harvest, samples of herbage were hand-separated and oven-dried to determine the botanical composition of each plot. Data on the percentage composition of grass, clover, and weeds are recorded in Table 2. No attempt was made to determine the exact proportion of each grass species, but it was apparent that orchard grass dominated, particularly at the higher rates. Brome was the next most evident grass species, and creeping red fescue the least. After the first harvest there were few weeds in any of the plots. Percentage clover remained approximately the same until the end of the first season, but the grasses increased to replace the weeds. These relationships held for all years in which the test was seeded except 1954. In the 1954 seeding, volunteer oat growth covered about 75 per cent of the plot area in an irregular pattern and made it impossible to distinguish among the effects of the different rates. Because of this interference, first-year results of the 1954 seeding are not reported.

The differences apparent in Figure 1 were similar for all years in which a seeding was made, and were reflected in the yields of the first harvest. Dry matter yields in the year of seeding for 1953, 1955, and 1956 are depicted graphically in Figure 2. In general, first-cut yields increased with the rate of seeding, reaching a maximum at a rate of about 30 pounds per acre. Second-cut yields were either unaffected by increasing rates of seeding or showed a slight decline.

TABLE 3.—SECOND-YEAR YIELDS OF FORAGE FROM PLOTS SEEDED AT SEVEN RATES IN 1953 AND EIGHT RATES IN 1954 AND 1955

Pounds of dry matter per acre

Rate per acre	1953 Seeding					1954 Seeding				1955 Seeding			
	Cut 1	Cut 2	Cut 3	Cut 4	Total	Cut 1	Cut 2	Cut 3	Total	Cut 1	Cut 2	Cut 3	Total
5 pounds	1720	840	600	280	3440	2320	2820	1300	6440	1520	920	3100	5540
10 pounds	2040	800	600	160	3600	1760	2360	1320	5440	1600	940	2920	5460
15 pounds	1920	740	400	160	3220	2160	2620	1360	6140	1560	960	2800	5320
20 pounds	2080	680	480	140	3380	2240	2440	1320	6000	1440	880	2840	5160
25 pounds	2000	620	340	80	3040	1960	2680	1380	6020	1520	840	2940	5300
30 pounds	1920	620	440	180	3160	1940	2400	1280	5620	1560	880	2800	5240
35 pounds	1840	580	320	80	2820	2380	2620	1400	6400	1580	900	2660	5140
40 pounds	—	—	—	—	—	2220	2560	1360	6140	1580	840	2420	4840
L.S.D. 5% level	220	120	180	80	340	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	360	N.S.

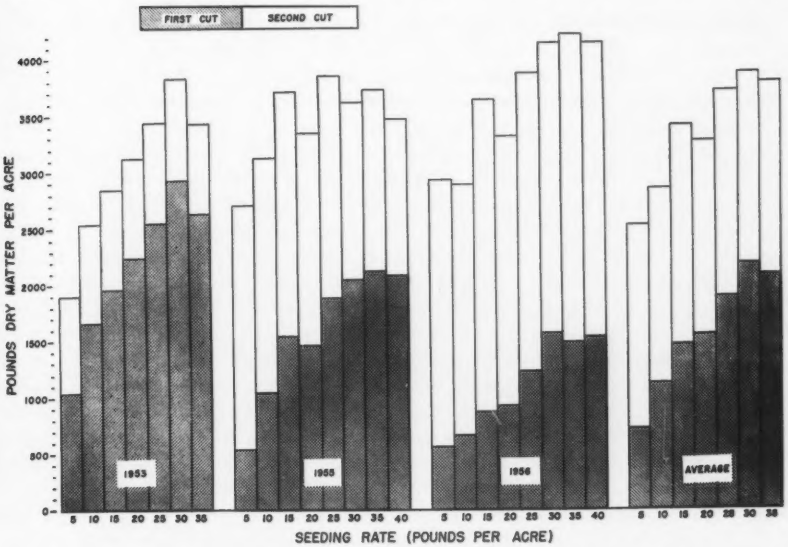

FIGURE 2. First-year yields of an irrigated pasture mixture seeded at seven rates in 1953 and eight rates in 1955 and 1956.

Plots of the 1953 to 1955 seedings were harvested three or four times in the year following seeding. Yields are recorded in Table 3. It can be seen from these data that second-year yields either remained unaffected by seeding rate, or tended to shift in favour of the lighter rates as the season advanced. Plant growth indicated a lack of soil nitrogen on these plots during the second year, and the shift in favour of the lighter rates probably could be attributed to depletion of fertility on the higher yielding plots of the previous year. An application of 250 pounds per acre of 16-20-0 was made in October of 1954, and may have been responsible for the higher 1955 yields of the 1954 seeding. A heavy application of nitrogen fertilizer was avoided because of its anticipated stimulation of grass species resulting in suppression of the clover.

Changes in botanical composition of the mixtures in the year following seeding involved mostly a levelling out of the proportions of clover on all plots. The general increase in clover probably was due to enlargement of existing plants rather than establishment of new ones. Data from 1956, summarized in Table 4, are typical of the trend of clover development that occurred each year.

DISCUSSION AND CONCLUSIONS

The data presented in this paper, and the work of Brougham (2) and Parry (5), indicate that pastures can be established by using lower seeding rates than those commonly employed. The data of Table 1 show that even at the 5-pound rate a large number of seedlings emerged per

TABLE 4.—PERCENTAGE CLOVER* AT THREE DATES IN 1956 OF A PASTURE MIXTURE SEEDED AT EIGHT RATES IN 1955

Rate of seeding per acre (lb.)	Percentage Clover in Mixture		
	Cut—May 30	Cut 2—June 26	Cut 3—August 14
5	12	19	35
10	11	18	40
15	9	16	40
20	10	17	47
25	8	16	44
30	5	12	40
35	7	11	42
40	6	10	41

* Based on oven-dry weights

unit area. Figure 2 shows that after the first harvest there was no yield advantage due to higher seeding rates. If cost of seed is a deterrent to the establishment of an irrigated pasture, the rate of seeding can be reduced.

However, cost of seed should not be the only criterion of economy of seeding rates. The time required for a sward to reach a stage suitable for grazing, and the increased yields from the heavier rates, though small and short-lived, are worthy of consideration. Irrigated land is valuable and should not remain unproductive any longer than is necessary. From field observations of both height of growth and sward density, it appeared that the heavier rates of seeding were ready for grazing from 2 to 4 weeks earlier than the lighter rates (Figure 1). Grazing pastures at this stage of development has been practised at Lethbridge without apparent injury to the plants. The feed value of this herbage usually will be in excess of the increased cost of seed. From the data presented it can be calculated that each 1-pound increase in seed from 5 to 30 pounds per acre resulted in an increased yield of 45 pounds of dry matter in the year of seeding. Except in times of very high seed prices and low prices for animal products, the herbage produced will have a higher value than the extra seed used.

The fact that in 1954 there was a tendency for yields to shift in favour of the lighter rates in the year following seeding is not regarded as important. As suggested earlier, the shift could be explained as depletion of fertility on the high yielding plots of the previous year. Such a shift probably would not occur under grazing conditions if the soil fertility were maintained at the high level demanded by good management of irrigated pasture.

Another important criterion of a suitable seeding rate is that it must produce the desired type of sward. In this experiment the best balance of species was produced by rates in the range of 15 to 25 pounds per acre.

Although data of Table 2 do not entirely substantiate the contention, rates lower than 15 pounds were much more subject to weed encroachment. The land used for this test was not seriously infested with weeds, so the problem was not as great as it might have been. The open ground in the 10 lb.-rate plots shown in Figure 1 probably would have been invaded by weeds if there had been a source of infestation. The heavier rates offer more competition to weed seedling establishment. Further, it should be pointed out that under flood irrigation thin stands do not provide sufficient soil protection against erosion. Rates of 30 pounds and higher produced swards with too high a proportion of orchard grass. Suppression of clover was one undesirable feature of the heavy rates. Clover suppression has been overcome under certain conditions, such as those reported by Sears *et al.* (6). They found that on pumice soils in New Zealand careful placement of phosphorous fertilizer at the time of seeding and high rates of *Rhizobium* inoculation gave more uniform emergence and better survival of clover seedlings. Under the conditions of this experiment, where good stands of clover are obtained normally, it appears that clover suppression is due to the aggressiveness of orchard grass at the higher rates of seeding. A reduction in the proportion of orchard grass in the seed mixture might hasten the development of the clover, but for the mixture tested a reduced seeding rate would appear to be effective.

The general conclusion is that the lowest rate of seeding that can be used to establish a pasture is not necessarily the most economical. These results are based on only one seed mixture, and one method of seeding, but the principles evolved should have general application.

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POLYSPORY IN AN AMPHIPLOID OF *TRITICUM-AGROPYRON* HYBRIDS[1]

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ABSTRACT

An amphiploid line in the F_9 generation tracing to the cross, *Triticum turgidum* × *Agropyron intermedium*, was found to contain plants which at meiosis showed a spindle abnormality. In plants showing this abnormality, over 50 per cent of the microsporocytes contained 5 to 8 microspores instead of the normal 4. The occurrence of microspores with two nuclei was also common and was more prevalent in tetrads than in hexads to octads. Meiosis was found to be regular up to metaphase II. At this time a secondary spindle was formed and the chromosomes divided and moved to the poles of primary or secondary spindles. The number of chromosomes on secondary spindles ranged from 3 to one-half the complement of 32-35. After telophase II wall formation was usually initiated enclosing the resulting nuclei; where walls failed to form microspores were two-nucleate. The result of this abnormality was to bring about almost complete sterility in the affected plants.

INTRODUCTION

Numerous cases have been reported of abnormalities at meiosis which are inherited in a mendelian manner. These abnormalities are mainly due to genes affecting chromosome pairing and less frequently to misfunction of the spindle mechanism. A case of polyspory in a *Triticum-Agropyron* hybrid line which was recently discovered in the breeding nursery, and which proved to be due to spindle misfunction, will be described here.

MATERIALS AND METHODS

The hybrid line in which polyspory was discovered originated from a cross, *Triticum turgidum* × *Agropyron intermedium*. The original hybrid was completely sterile but was converted into a fertile amphiploid by colchicine treatment. The cytological and breeding behaviour has been described by Armstrong and White (1). Several F_9 lines were subjected to cytological examination to determine the chromosome number and chromosome association at first metaphase. Observations on line S55-31 are reported herein.

The object of the cytological study was to aid the plant breeder by determining the chromosome number and studying the chromosome associations at first metaphase. Anthers at approximately metaphase I were collected from five plants of S55-31, fixed in absolute-acetic (3:1) and stored in vials in a refrigerator for 2 or 3 months. Squash preparations were then made with aceto-carmine. The slides were made permanent with euporal.

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TABLE 1.—CHROMOSOME ASSOCIATIONS AT FIRST METAPHASE

Generation	Year studied	Breeding No.	2n number	Association of chromosomes			
			1	2	3	4	
F_9	1957	S55–31–1	66	4.0	29.6	.3	.3
		–2	66	4.6	28.4	.2	1.0
		–3	68	5.2	31.0		.2
		–4	70	3.0	33.5		
		–5	70	4.8	34.6		
	Mean		68.0	4.3	31.4	.1	.3
F_8	1954	S53–35–3	64	2.0	31.0		
		–5	64	1.8	30.8	.2	
		–7	66	4.0	31.0		
		–9	62	4.0	29.0		
			64.0	2.9	30.4	.1	
F_6	Mean 1950	11 plants from mass population	62.5	5.4	25.8	.34	.10

OBSERVATIONS

Three of the five collections contained anthers at the tetrad stage and the abnormality occurred in two of these. Typical polyspores are illustrated in Figures 10-12. Instead of the normal tetrads of 4 microspores about 50 per cent consisted of complexes of cells composed of 5 to 8 microspores. A study was made of all available stages to determine the cause of the abnormality.

The cytological behaviour of the plants in line S55-31 at metaphase I proved to be quite typical of the plants of this amphiploid. Table I summarizes the chromosome associations at first metaphase for the five plants. For comparison four plants in the previous generation are included. A group of 11 plants selected at random from the F_6 generation shows the average behaviour for this hybrid material. This random group of plants averaged 62.5 chromosomes per plant representing a mean chromosome loss from the initial amphiploid of 7.5. The cytological condition was still unstable, multivalents and univalents being fairly frequent. From F_7 forward, line selection was practised instead of general mass increase and plants with higher chromosome numbers were used to establish the lines. The F_8 plants as might be expected have a higher mean chromosome number than the F_6 population and have a somewhat lower incidence of univalents and multivalents. While the F_8 parent of the S55-31 plants was not examined cytologically the progeny suggest that it may have been a 68-chromosome plant.

Based on metaphase behaviour the line, S55-31, would be given a very favourable rating for regularity. Departure from the original amphiploid number of 70 is very slight and bivalent pairing is the rule with multivalent and univalent frequency fairly low. It is quite evident that the abnormal condition observable at the tetrad stage is not due to factors

TABLE 2.—FREQUENCIES OF CLASSES OF POLYSPORES AND NUCLEAR CONDITION IN ABNORMAL PLANTS.

	No. of microspores in polyspore complex					Per cent with more than 4 microspores
	4	5	6	7	8	
	Plant	55–31–4				
No. of polyspores	18	17	12	3	2	65.4
No. of microspores	72	85	72	21	16	
Per cent binucleate	13.8	7.1	1.4	5.0	6.6	
	Plant	55–31–5				
No. of polyspores	20	12	7	3	1	52.2
No. of microspores	80	60	42		8	
Per cent binucleate	63	1.8	2.4	3.4	0	

affecting chromosome pairing. Typical cells at metaphase I are shown in Figures 1 and 2. The bivalents disjoin normally and proceed to the poles in what appears to be normal movement. Figure 3 shows a typical anaphase with a well stained spindle. The spindle indicates good convergence at the poles.

Following the second division which normally yields a tetrad of microspores, the abnormality makes itself readily apparent. While the homotypic division is regular in many cells (Figures 4 and 5), there is a high proportion of microsporocytes showing 5-8 cells. Table 2 shows the frequency of cell complexes composed of 4, 5, 6, 7 and 8 microspores in the two abnormal plants. The frequencies are quite similar for the two plants.

Many of the spores were characterized by two nuclei. The percentages of cells with two nuclei for the various polyspore classes is included

Meiosis in abnormal line. (These photomicrographs have been reproduced at a magnification of approximately × 1000).

FIGURE 1. First metaphase of plant 55-31-4 showing normal pairing except for the occurrence of a low frequency of univalents which are not associated with the main features in abnormal division. Occasional multivalents also occur.

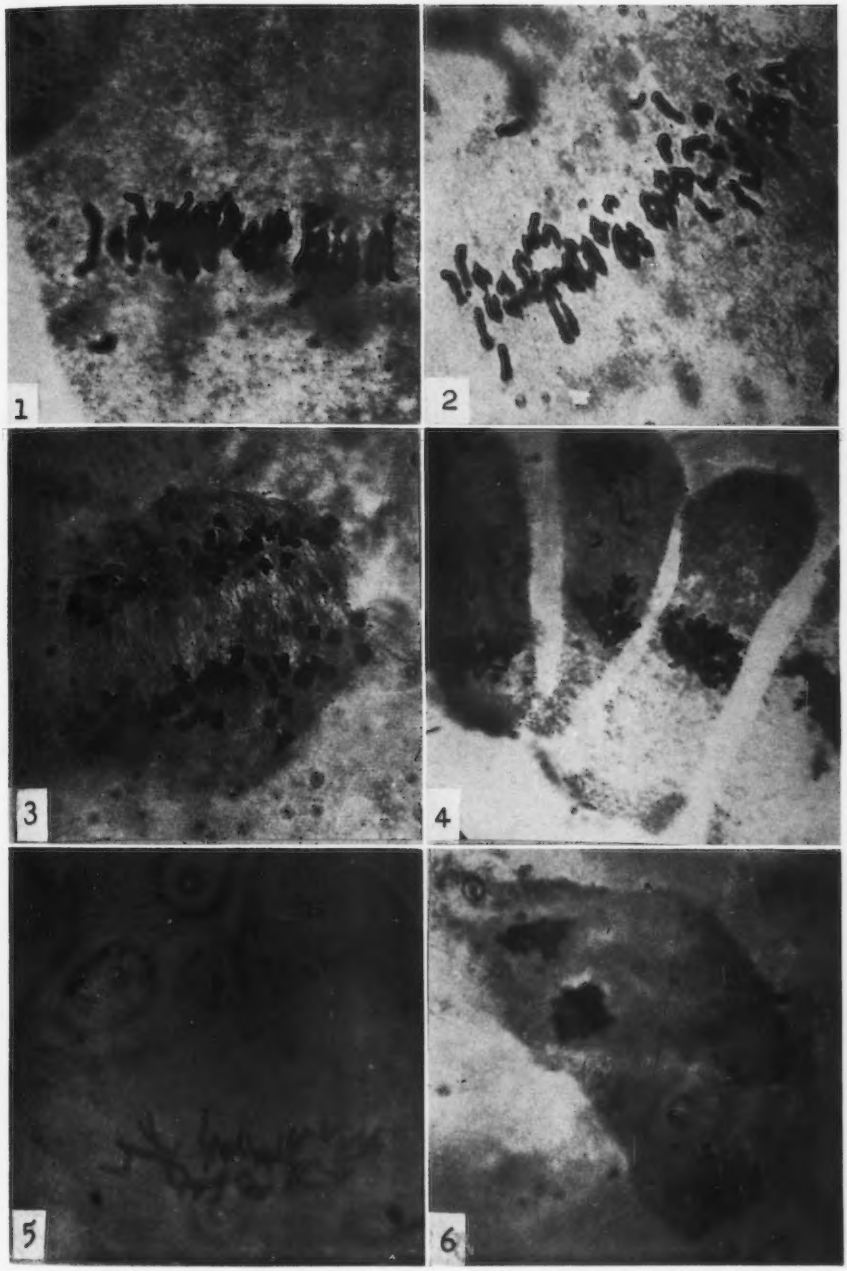
FIGURE 2. Beginning of first anaphase in 55-31-4. Separation of bivalents is normal. Univalents divide homotypically and reach the poles of cell in time to be included in the resting nucleus.

FIGURE 3. Mid-anaphase of 55-31-4 showing regular division of bivalents; the spindle appears quite normal with no suggestion of bipolar split.

FIGURE 4. Normal second division cells. Figure to left shows dyads at metaphase II. In figure to right the dyads are at late anaphase II. Univalents if not included in the main nucleus in the resulting tetrad are present in the cytoplasm as micronuclei.

FIGURE 5. Normal second division. The direction of arms of chromosomes suggest movement toward single poles.

FIGURE 6. Abnormal dyad showing four groups of chromosomes at telephase II. The groups are approximately equal in size and should eventually yield four microspores.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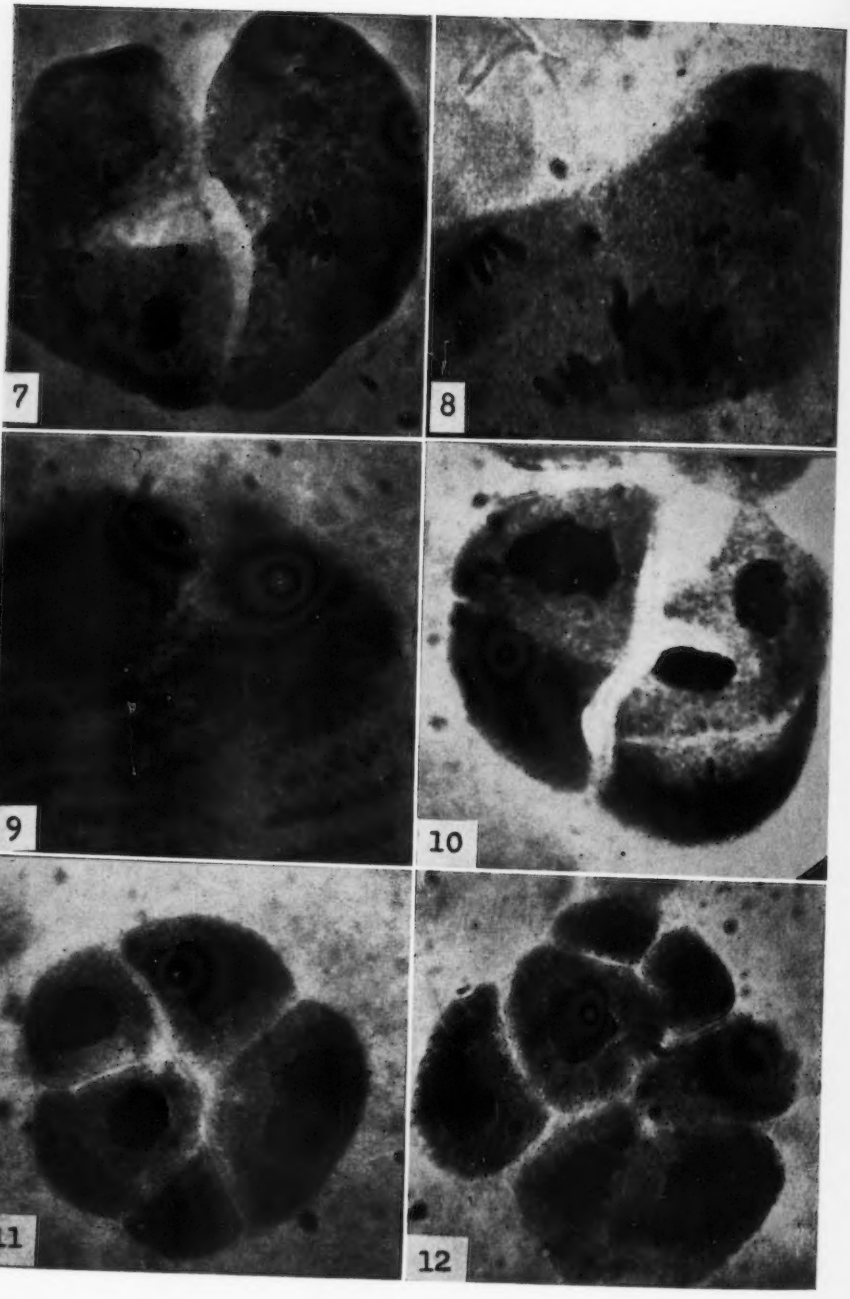
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in Table 2. The tetrad type of polyspore shows a higher frequency of binucleate cells than do the higher complexes. Figure 10 shows a tetrad in which one microspore has two nuclei. It may be seen that wall formation is being initiated which would eventually produce a pentad of microspores.

The abnormal microspore condition appears to be the result of spindle misfunction in the second division. Figures 6, 8 and 9 illustrate this. Figure 6 shows a dyad in which the chromosomes are moving to the poles on two separate spindles. In this case the resulting nuclei appear to be about equal in size. In Figure 8 the larger portion of the chromosomes have divided and are moving to the poles on the main spindle. A smaller group of three chromosomes have divided, and are being oriented on an independent spindle. In Figure 9 (only one end of the cell is in focus) one dyad is bipolar at one end and tripolar at the other end. This would eventually produce a pentad of microspores. This unequal division of chromosomes on separate spindles may be clearly inferred by examining the polyspore complexes. The size of the nuclei in them varies considerably due to varying chromatin masses.

In Figure 7 an interphase between the two divisions is shown. There appears to be a breaking-up of the chromosomes into groups. It is possible such groups may initiate separate spindles. However, this type of interphase was noted very infrequently and cannot be considered the sole cause of multiple spindle formation.

The behaviour of univalents in this material is typical of many hybrid species and is quite independent of the main abnormality. Thus univalents divide equationally at the first division and lag behind the normal bivalents as they move to the poles. They fail to divide at the second division and have no spindle formation but are usually included in the nuclei of the microspores. If they are not included they form micronuclei in the cytoplasm. Such micronuclei may be seen in Figure 12. No case was noted where the micronuclei were separated by cell walls and might be termed microspores.

This abnormality might be expected to have a marked effect on seed production. This assumption would be true if the course of megasprogenesis was disturbed. At harvest time heads were gathered from this

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FIGURE 7. In this cell at metaphase II the chromosomes are divided into groups each of which may initiate its own spindle. In the dyad to the left there is a suggestion of wall formation.

FIGURE 8. Abnormal second telephase. The main group of chromosome have divided and separated on one spindle. A small group of 3 chromosomes have a separate spindle. This would lead to 2 large and 2 small microspores.

FIGURE 9. Microsporocyte in which one dyad has divided normally. In the other dyad there are 3 nuclei at one end of cell and 2 at other.

FIGURE 10. Tetrad in which one microspore contains 2 nuclei. A wall is forming between these 2 nuclei which will eventually convert tetrad to pentad.

FIGURE 11. Heptad of microspores. The size of cells and direction of dividing lines suggest that 1 dyad divided normally while the other had a double spindle which lead to four equal sized microspores.

FIGURE 12. Septad of microspores. Both dyads had presumably abnormal spindle formation.

line and sampled for fertility. The fertility of 27 heads ranged from 0 to 8 seeds per head with an average of 1.6. Since the average fertility for this amphiploid was 20 seeds per head the fertility of the abnormal line is about one-tenth of normal.

DISCUSSION

Since meiosis in this irregular line appears normal in all cells in the first division, the abnormality is not due to a gene affecting metaphase pairing. Its obvious effect is to produce polyspores. They in turn trace to misfunction of the spindle at the second division.

Abnormal spindle behaviour appears to have two distinct results or to be of two types. One type leads to an increase in the number of chromosomes in the microspores and the other to a reduction of the normal number. Typical of the former is the example from *Kniphofia* reported by Moffat (7). Spindle failure was found to occur in either the first or second division with subsequent failure to form a wall. As all cells were not affected microspores might contain n, 2n, or 4n chromosomes. Dowrick (5) reports a fairly similar case in *Pyrus* where failure of wall formation at the tetrad stage was considered as a contributing cause of polyploidy. Typical of the second type of spindle irregularity was that reported by Clarke (3) in maize and Darlington and Thomas (4) in a *Festuca-Lolium* derivative. Spindle irregularity was noted at first anaphase; the chromosomes diverging into groups at the interphase instead of converging to form a single nucleus. Since each group formed its own spindle in the second division finally four to several microspores were formed from each PMC.

The case reported here belongs to the second type with some modification. All first anaphases observed appeared to be typical of that shown in Figure 3, where the spindle is clearly of the converging type. It appears logical to assume that a similar type of gene might be operative here to that found in maize, with its action or expression delayed until the second division. There is a suggestion from Figure 7 that occasionally this gene may become active sooner in a proportion of cells causing a grouping of chromosomes at interphase.

A factor equally important to that of the spindle in producing this polysporus condition is wall formation. Belar (2) showed that spindle behaviour and wall formation are closely related. If the former failed in its function of contracting and compacting the chromosomes at the poles of the cell, walls were not laid down. In Table 2 there is seen to be a definite relationship between the degree of polyspory and the number of nuclei per microspore. There is a higher percentage of tetrads with two nuclei than in the classes pentads to octads. In some cases where a microspore is binucleate the wall cleavage has started. This is apparent in Figure 10. It is possible that each binucleate microspore would eventually be converted into two microspores if the process had not been halted by the fixation of the material.

A further indication that the cell mechanism which controls spindle formation also controls wall formation is shown by univalent behaviour. In our material, which is typical of most hybrids, the univalents divide at the first division but are not motivated to the poles at the second division by spindle action. If they fail to be included in the nuclei at telephase II they remain in the cytoplasm as micronuclei. In the *Viola* hybrids reported by Manch (6), in the homotypic division the univalents formed their own spindles and divided and separated regularly. Nuclear membranes form about the single chromosomes and wall formation took place.

From the limited amount of material examined in this study it is impossible to determine the nature of the genic control exercised by the factor or factors responsible for the spindle abnormality. In this case and in several others reviewed, the irregularity is apparent in approximately half of the tetrads which would suggest that the agency of normal or abnormal chromosome division resides in the chromosomes themselves. At the tetrad stage it would appear that one dyad frequently has divided normally while the other produced three or more microspores. A single bivalent carrying the genes in the heterozygous condition could conceivably exercise control over spindle-forming activity.

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THE ROLE OF FERTILIZER AND 2,4-D IN THE CONTROL OF PASTURE WEEDS[1]

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ABSTRACT

A study was made of: 1) the response of some pasture weeds of Eastern Canada when fertilizers were applied; 2) the productivity of pastures sprayed with 2,4-D, and 3) the over-all response when both fertilizer and 2,4-D were applied. Populations of orange, yellow and mouse-ear hawkweeds, ox-eye daisy, chicory, bugleweed, strawberry and wild carrot were decreased when nutrients were added but the numbers of dandelion, Canada thistle, tall buttercup, shore horsetail and yarrow were unchanged. Fertilizer not only improved the quantity but also the quality of the forage by reducing the weed content. Yield of total vegetation was not increased when weeds were controlled by 2,4-D but the forage consisted almost exclusively of desirable grasses. Best results from the standpoint of both yield and absence of weeds were obtained when fertilizer treatment was supplemented by applications of 2,4-D.

INTRODUCTION

The abundance of some weeds in pastures is related to the fertility of the soil. It has been known for a long time (1, 3, 4, 8) that weeds are more numerous if soil fertility is low, and that total weed populations can usually be reduced by correcting the pH and adding nutrients. On the other hand, Shuh (7) has observed that some weeds such as Canada thistle (*Cirsium arvense* L.), bull thistle (*C. vulgare* (Savi) Tenore), tansy ragwort (*Senecio jacobaea* L.) and tall buttercup (*Ranunculus acris* L.) appear to invade or, at least, persist in fertile pastures. Klingman (5) in a recent review on pasture weed control suggested further that some perennial weeds may even benefit from the application of fertilizer in pastures.

The herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid) is recommended for the control of many broadleaf weeds in pastures (3). However, there is some question as to whether or not the use of a herbicide alone leads to greater yields in pastures where fertility is the factor limiting production.

This paper presents data on the response of some pasture weeds of Eastern Canada when fertilizer is applied, on the yields of the forage from pastures sprayed with 2,4-D, and on the over-all response when both fertilizer and 2,4-D are applied together.

MATERIALS AND METHODS

Fertilizer and Liming Trials

In the fall of 1951, limestone at 0, 2, 4 and 6 tons per acre was applied to plots laid out on De l'Anse clay loam near Ste. Anne de la Pocatière, Quebec. This land is poorly drained, high in organic matter and has a pH of 4.8. Fertilizer (300 lb. of 2-16-6 per acre) was applied at seeding time

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in 1952 and also in the spring of 1954 and 1956. A mixture of timothy, alsike, red clover and alfalfa was seeded down in 1952 with oats as the nurse crop. The forage was cut twice a year from 1953 to 1955 and four times a year from 1956 to 1958, but the area was never grazed. Plots were 1/200 acre in size and each treatment was replicated four times. Counts (two per plot) of the different weed species were made in 1958.

Fertilizer and 2,4-D Trials

In the spring of 1956, plots 20 feet square were laid out in two permanent pastures near Ottawa. One set of plots was on Carp clay loam (pH 6.4, organic matter 7 per cent, cation exchange capacity, 25 milli-equivalents per 100 grams) while the other was on Grenville loam (pH 7.3, organic matter 5 per cent, cation exchange capacity, 19 milli-equivalents per 100 grams). Each treatment was replicated four times at each site. Treatments were as follows:

1. Check, no treatment.
2. Fertilizer applied each year at 50–60–90 pounds per acre of N–P_2O_5–K_2O, respectively. One-half of the P_2O_5 and K_2O was applied in the fall and the remainder in the spring of the following year, starting in fall of 1956. All of the nitrogen was applied in the spring.
3. Butyl ester of 2,4-D applied at 1 pound per acre in June and early September of each year starting in June 1956.
4. A combination of treatments (2) and (3).

Square yard cages were set in the plots so that forage samples could be harvested but otherwise the areas were continuously grazed. When the second cut was taken in 1958, the weeds were separated out and weighed independently to determine the percentage of weeds in the forage. Counts (not less than 5 per plot) of the different weed species were made periodically.

TABLE 1.—EFFECTS OF LIMESTONE AND FERTILIZER ON WEED POPULATION ON DE L'ANSE CLAY LOAM.

Weeds	Number of plants per square yard								L.S.D. 0.05
	No lime		2 tons lime		4 tons lime		6 tons lime		
	Check	Fert.	Check	Fert.	Check	Fert.	Check	Fert.	
Orange hawkweed	398	367	252	136	108	63	105	77	51
Mouse-ear hawkweed	51	14	16	0	34	3	11	0	14
Ox-eye daisy	20	35	16	7	10	0	4	4	13
Bugleweed	188	90	152	55	60	4	67	10	39
Virginia strawberry	11	17	3	0	0	2	0	0	7
Sub-total	668	523	439	198	212	72	187	91	
Dandelion	47	50	53	40	72	50	55	69	N.S.
Shore horsetail	34	41	29	19	24	35	21	29	N.S.
Yarrow	9	12	15	8	8	19	10	15	N.S.
Sub-total	90	103	97	67	104	104	86	113	
TOTAL	758	626	536	265	316	176	273	204	

Kentucky blue grass (*Poa pratensis* L.) and wild white clover (*Trifolium repens* L.) were the desirable species on the Carp clay loam whereas Kentucky blue grass was the predominant forage species on the Grenville loam. The main weeds at all sites were tall buttercup (*Ranunculus acris* L.), northern bugleweed (*Lycopus uniflorus* Michx.), wild carrot (*Daucus carota* L.), chicory (*Cichorium intybus* L.), Canada thistle (*Cirsium arvense* (L.) Scop), dandelion (*Taraxacum officinale* Weber), orange hawkweed (*Hieracium aurantiacum* L.), mouse-ear hawkweed (*H. pilosella* L.), yellow hawkweed (*H. florentium* All.), shore horsetail (*Equisetum litorale* Kuhlewein), ox-eye daisy (*Chrysanthemum leucanthemum* L. var. *pinnatifidum* Lecoq and Lamotte), Virginia strawberry (*Fragaria virginiana* Duchesne) and yarrow (*Achillea millefolium* L.).

EXPERIMENTAL RESULTS

Fertilizer and Liming Trials

Results in Table 1 show that the total weed population was decreased when limestone and fertilizer were applied to De l'Anse clay loam. However, not all species responded in a similar manner. The populations of orange hawkweed, mouse-ear hawkweed, ox-eye daisy, bugleweed and strawberry

TABLE 2.—THE RESPONSE OF INDIVIDUAL WEED SPECIES TO 2,4-D AND FERTILIZER IN A PASTURE ON GRENVILLE LOAM

	Number of plants per square yard			
	Yellow hawkweed	Ox-eye daisy	Wild carrot	Total
Check	164.0	3.6	28.0	195.6
Fertilizer	26.0*	1.7	15.1*	42.8
2,4-D	0.0**	0.4*	0.0**	0.4
2,4-D plus fertilizer	0.0**	0.1*	0.5**	0.6
L.S.D. .05	58.8	2.3	13.0	

*Differences significant at 5% level
**Differences significant at 1% level

TABLE 3.—THE RESPONSE OF INDIVIDUAL WEED SPECIES TO 2,4-D AND FERTILIZER IN A PASTURE ON CARP CLAY LOAM

	Number of plants per square yard				
	Chicory	Dandelion	Canada thistle	Buttercup	Total
Check	6.4	90.0	2.5	3.8	103.7
Fertilizer	3.6*	83.0	2.5	2.9	96.1
2,4-D	0.0**	1.8**	0.4**	3.3	5.5
2,4-D plus fertilizer	0.0**	1.8**	0.7**	2.3	4.8
L.S.D. .05	2.0	23.7	0.3	N.S.	

*Differences significant at 5% level
**Differences significant at 1% level

decreased when limestone alone was applied and decreased still further when fertilizer was added as well. On the other hand, the number of dandelion, shore horsetail and yarrow remained constant irrespective of the treatment.

Fertilizer alone decreased the number of mouse-ear hawkweed and bugleweed, but did not appear to affect the orange hawkweed, ox-eye daisy and strawberry unless it was combined with the limestone. The population of ox-eye daisy was increased by fertilizer alone and decreased by the limestone. No additional control of weeds was achieved when more than 4 tons per acre of limestone were applied.

Fertilizer plus 2,4-D Trials

On the Grenville loam, yellow hawkweed, ox-eye daisy and wild carrot were the predominant weed species. As shown in Table 2, the fertilizer alone caused significant reductions in the population of yellow hawkweed and wild carrot but not of ox-eye daisy. Each of these weeds was virtually eliminated by 2,4-D alone.

Dandelion, tall buttercup, chicory and Canada thistle were the predominant weeds on the Carp clay loam site. Table 3 shows that, although the population of chicory was reduced by the fertilizer alone, this treatment had no effect on the population of dandelion, tall buttercup or Canada thistle. The 2,4-D alone was very effective in controlling chicory and dandelion and brought about a marked reduction in the stand of Canada thistle. Tall buttercup, however, was not affected by any of the treatments. With none of these weeds was the control obtained by 2,4-D improved when it was combined with fertilizer.

Table 4 shows the dry weight of the weeds and of the total forage produced at the time of the second cut in the summer of 1958. While samples from the check plots at the two sites consisted of 72 and 87 per cent weeds, those from the plots treated with 2,4-D contained only traces of weeds. The samples from the plots which received the fertilizer alone consisted of only 21 and 17 per cent weeds. This change could be attributed partly to the increase of the desirable species but the data also show that there was an actual decrease in the dry weight of the weeds produced, likely due to increased competition.

Yield data for the tests on Carp clay loam and Grenville loam are recorded in Tables 5 and 6. The yield totals for each year at the two sites were analysed separately. In no case did the application of 2,4-D alone

TABLE 4.—EFFECT OF 2,4-D AND FERTILIZER ON THE YIELD AND PER CENT OF WEEDS IN FORAGE ON TWO SOIL TYPES, SECOND CUT, 1958

	Grenville loam				Carp clay loam			
	lb. DM/A			% Weeds	lb. DM/A			% Weeds
	Forage	Weeds	Total		Forage	Weeds	Total	
Check	72	442	514	87	323	813	1136	72
Fertilizer	600	125	725	17	1410	397	1807	21
2,4-D	530	trace	530	—	1293	trace	1293	—
2,4-D plus fertilizer	949	trace	949	—	2471	trace	2471	—

TABLE 5.—EFFECTS OF 2,4-D AND FERTILIZER ON YIELDS OF FORAGE ON GRENVILLE LOAM

	Forage yields in lb. dry matter per acre					
	1957			1958		
	1st cut	2nd cut	Total	1st cut	2nd cut	Total
Check	485	645	1130	219	514	783
Fertilizer	1072	685	1757	1889	725	2614**
2,4-D	555	477	1032	477	530	1007
2,4-D plus fertilizer	1411	834	2245	2487	949	3436**
L.S.D. .05			N.S.			551

**Differences significant at 1% level

TABLE 6.—EFFECTS OF 2,4-D AND FERTILIZER ON YIELD OF FORAGE ON CARP CLAY LOAM

	Forage yields in lb. dry matter per acre						
	1957				1958		
	1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	Total
Check	568	998	927	2493	2028	1136	3164
Fertilizer	1449	1107	1335	3881**	3406	1807	5213*
2,4-D	475	704	541	1720*	1661	1293	2954
2,4-D plus fertilizer	1502	893	1135	3530**	2494	2471	4965*
L.S.D. .05				648			1677

*Differences significant at 5% level
**Differences significant at 1% level

result in yield increases. Nor, (with the exception of the 1958 results on Grenville loam), did it increase the total yields when applied with fertilizer. The data for 1957 on the Carp clay loam showed a decreased yield when 2,4-D alone was applied. This was probably due to the fact that many of the weeds were eliminated. The white clover was injured but since the yields in 1958 were not depressed where 2,4-D was applied, this injury did not seem to affect the total yield.

Although the 2,4-D did not increase the yield of total vegetation produced, it had a very profound effect on the quality of the forage since the plots which received the 2,4-D contained only a trace of weeds while the check and fertilizer alone consisted of 80 and 20 per cent weeds respectively.

DISCUSSION AND CONCLUSIONS

It is clear from the results in Tables 1, 2 and 3 that not all the weed species responded in a similar manner when nutrients were added to pastures. These data confirm the observations of Shuh (7) and Klingman (5) that some weeds will persist even when the fertility of the soil is raised and point out the importance of studying the response of individual weed species rather than merely the total weed population as was done by many

of the early workers. Fertilizer alone had no effect on the population of dandelions, shore horsetail, yarrow, Canada thistle and tall buttercup, whereas marked decreases of mouse-ear hawkweed, yellow hawkweed, chicory, bugleweed, and wild carrot were obtained. On the acid soil, the control of orange hawkweed, ox-eye daisy and strawberry was not affected by the fertilizer unless applied in conjunction with the limestone. Thus, for many species of weeds the addition of fertilizer is sufficient to effect control but for other species different measures will be necessary.

The control of yellow hawkweed, ox-eye daisy, wild carrot, chicory and dandelion by 2,4-D was excellent. Since the plots were treated five times over a period of 3 years, regrowth from partially killed plants and from new seedlings was destroyed by the follow-up applications. Accordingly, better results were obtained than could be expected from a single treatment. Canada thistle is considered to be relatively resistant to 2,4-D because of the difficulty in obtaining a satisfactory kill of the roots. These data show, however, that with repeated applications the population of Canada thistle plants in pastures can be greatly reduced. Although some strains of wild carrot have been reported to be resistant to 2,4-D, excellent control of this species was obtained in this test. Since no control of tall buttercup was obtained it must be concluded that this species is resistant to 2,4-D.

The yield data from these tests support the hypothesis that total yields of forage are not increased when weeds are controlled by 2,4-D, and that yield increases result only when fertility is raised. The fertilizer alone not only improved the quantity but also the quality of the forage by partially reducing the weed content. The best results from the standpoint of both yield and absence of weeds were obtained when the fertilizer treatment was supplemented by 2,4-D. Although the herbicide injured the white clover this did not affect the final yield which was almost completely made up of Kentucky blue grass. These results agree with those of Klingman and McCarty (6) who found that when broadleaf weeds were controlled by 2,4-D the total production of vegetation did not change but there was an increase in basal density of the desirable grasses.

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INVESTIGATIONS ON CURING BURLEY TOBACCO WITH ARTIFICIAL AIDS[1]

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ABSTRACT

Two methods of providing supplementary aid to natural curing of cigarette burley tobacco were investigated through five consecutive curing seasons. In one method temperature and humidity controlled heaters were used, and in the other a specially constructed calcium chloride dehydrator. Both methods were compared concurrently with natural air curing in respective pilot barns of three-quarter acre capacity. A similar comparison of curing was made in heated and unheated 3-acre capacity barns through two curing seasons.

Supplementary heating produced the best results (improved quality, increased yields) but was ineffective during exceptionally cool weather.

INTRODUCTION

The periodic occurrence of high atmospheric moisture in burley tobacco barns is generally recognized as the primary cause of badly cured tobacco. It has been found that high quality burley tobacco can be cured at temperatures between 60°F. and 90°F. if the average relative humidity is maintained between 65 and 70 per cent (1, 2, 3). It has also been found that an increase or a decrease in relative humidity of 10 per cent from this optimum range reduces the yield of cured tobacco significantly (1). At Harrow, the temperature and the average relative humidity are usually within the optimum range but the relative humidity within tobacco barns is generally much higher particularly at night. It was, therefore, decided to investigate various means for reducing relative humidity in the curing barns. Two practical methods were tested: 1) The use of regulated heat, and 2) the use of a desiccant. The results of these tests are reported in this paper.

MATERIALS AND METHODS

Three pilot barns, each 28 feet square by 28 feet in height, with capacity for approximately three-quarters of an acre of tobacco, were constructed at the Research Station, Harrow, Ontario. These barns were similar to those in general use but were much smaller. The reduced size provided for sufficient bulk of tobacco to induce the inherent problems of air curing without the undue difficulties involved in handling larger crops. Another deviation from standard construction was the provision of a cupola-type ventilator extending the full length of the ridge which could be opened or closed at will. This feature was considered necessary for the heat-curing procedure and was therefore installed on all barns for the sake of uniformity. One barn was equipped with nine propane gas heaters which were automatically regulated to high or low flame intensity by adjustable humidistat and thermostat controls which operated independently. The heaters, which had a combined maximum capacity of 270,000 B.T.U., were spaced

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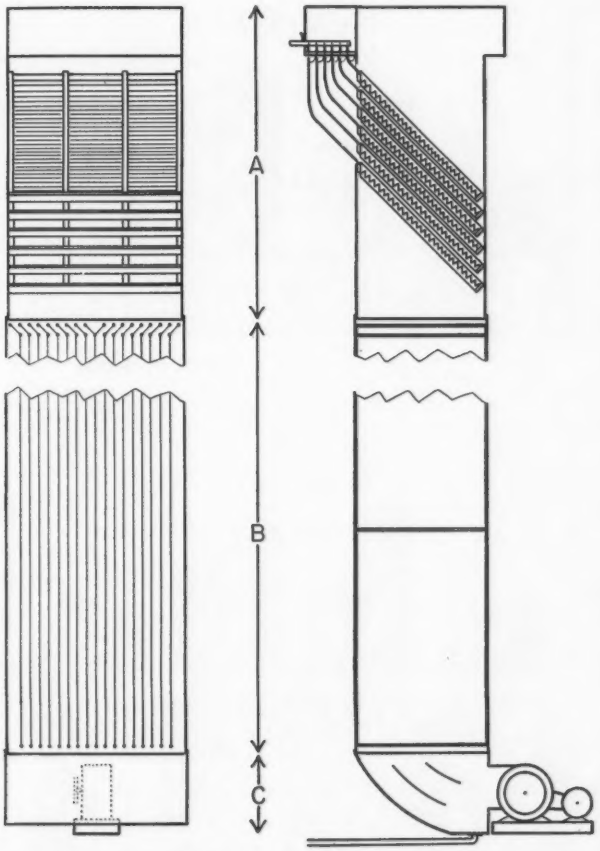

FIGURE 1. A diagrammatic illustration of the calcium chloride dehydrator. Approximate dimensions, height 24 feet, width 40 inches, depth 30 inches. Maximum absorption capacity, 250 pounds of water per 100 pounds of calcium chloride in 24 hours.

Section A. Illustrates the arrangement of the filling hopper and six corrugated copper screen trays to carry the calcium chloride flake.

Section B. Contains 19 linen wicks, each 16 feet long by 31 inches wide, to carry the calcium chloride brine descending from the trays.

Section C. Shows the arrangement of the air blower and plenum chamber where the air enters the dehydrator and the location of the brine drain-off pipe.

uniformly on the barn floor beneath the tobacco. The electrically operated humidistat and thermostat controls were located amidst the tobacco. Another barn was equipped with a calcium chloride dehydrator designed by the author, according to principles determined by preliminary studies of this type of equipment for curing burley tobacco. (Figure 1). The dehydrator was installed inside the barn adjacent to the end wall and extended from the floor to the upper part of the gable. A door was provided in the gable and on the outside a balcony was located from which the

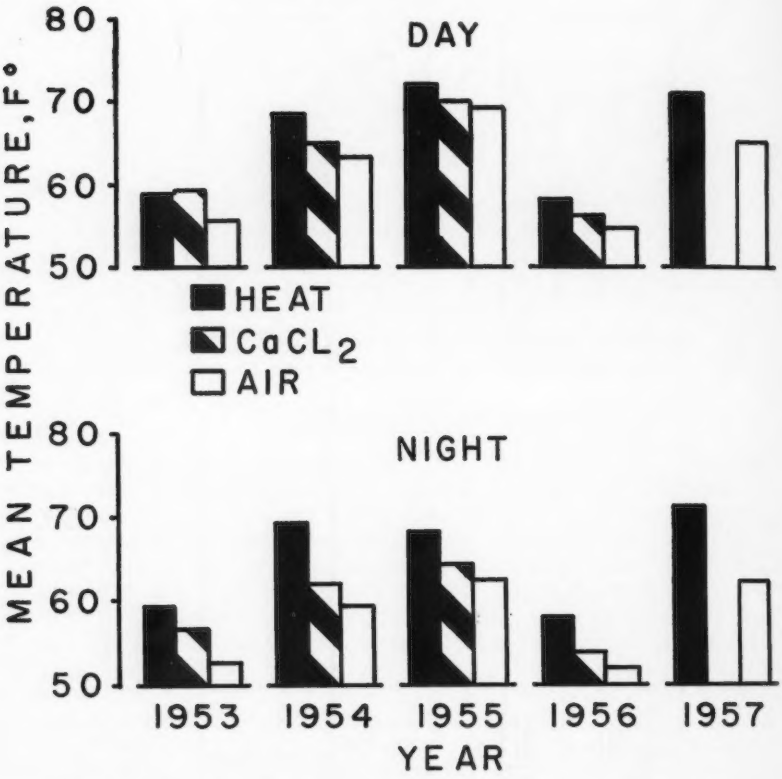

FIGURE 2. Mean annual temperatures by day and night periods for heat, calcium chloride, and air curing.

hopper was filled with calcium chloride flake as required. The calcium chloride was elevated to the hopper in 100-pound bags by means of a pulley hoist. In operation the fan at the bottom of the dehydrator recirculated the air upwards through the dehydrator and downwards through the tobacco at the approximate rate of 5000 c.f.m. The maximum absorption capacity of this unit was about 250 pounds of water per 100 pounds of calcium chloride in 24 hours. The third barn, which served as the control in these investigations, contained no special equipment to assist curing.

The procedure adopted for 16 days immediately after filling the barns each year with cigarette burley tobacco was as follows: The ventilators on all three barns were open during the day in fair weather and were closed at night and at any other time when rain or damp weather occurred. At such times, night or day, when the ventilators were closed, the propane burners in the heated barn were ignited and the ridge ventilator was opened to provide an outlet for the warm, moisture-laden air. However, on

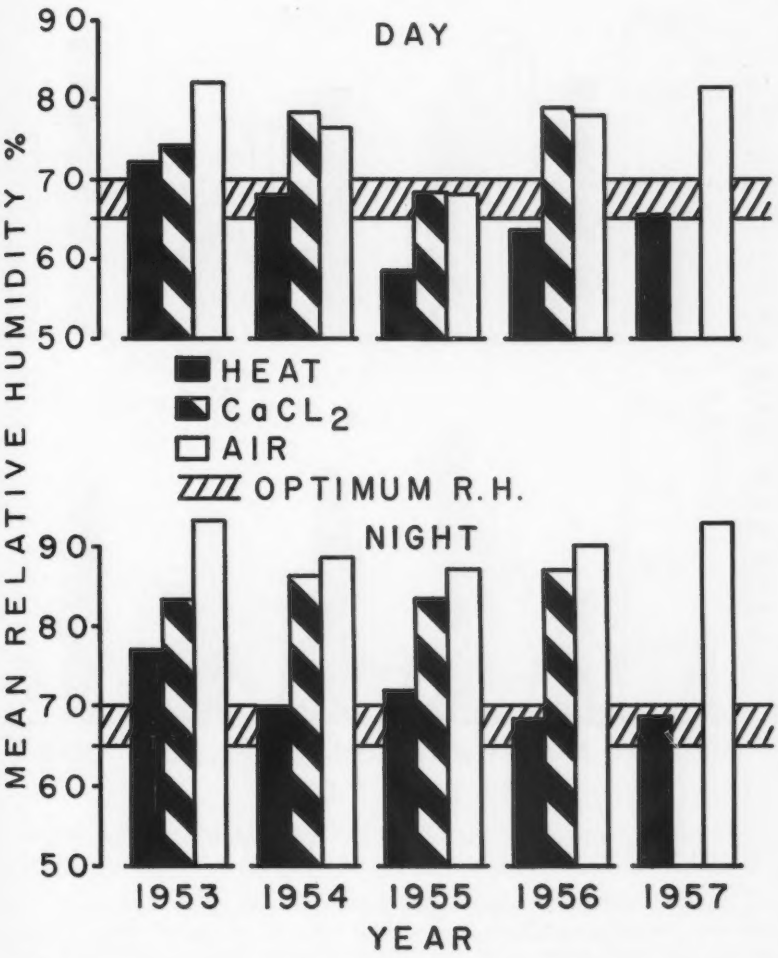

FIGURE 3. Mean annual relative humidities by day and night periods for heat, calcium chloride and air curing.

occasion when it was estimated in early evening that the tobacco was sufficiently dry and the prospects were for dry weather to continue during the night, the burners were not lit. The humidistat was set to increase flame level if the relative humidity exceeded 68 per cent and the thermostat was set to increase flame if the temperature fell below 60°F. When the ventilators were closed on the barn equipped with the calcium chloride dehydrator, the circulating fan was started and the dehydrator was kept charged with calcium chloride flake as required.

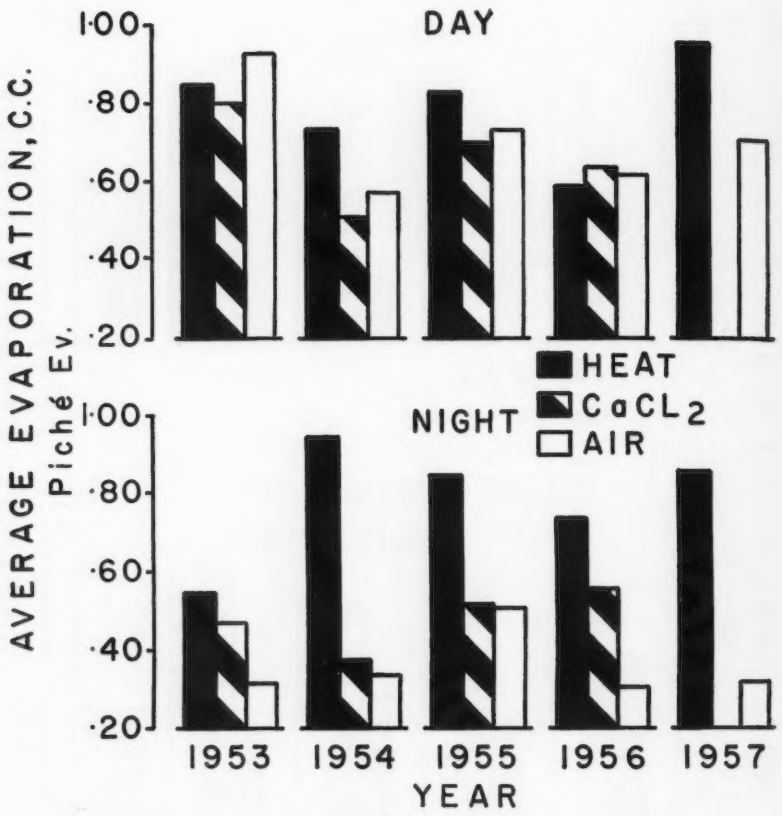

FIGURE 4. Average annual evaporation by day and night periods for heat, calcium chloride and air curing.

Several methods were employed to measure and assess the effects of the different curing procedures. Since natural air curing conditions differ greatly between night and day, the effects on curing conditions were compared by night and day periods respectively. The mean temperature and mean relative humidity data were obtained with long-distance recording psychrometers. A measurement of the physical effects on drying was obtained from morning and evening readings of Piché evaporimeters, while the actual amount of evaporation from tobacco at the same time was obtained from weight loss records of given samples of tobacco. The decrease in weight of tobacco was assumed for the purpose of this study to represent a sufficiently accurate measure of the evaporation of moisture from tobacco since the dry matter loss is small in relation to the vast quantity of water removed during curing. Finally, the tobacco was graded for quality and weighed for yield.

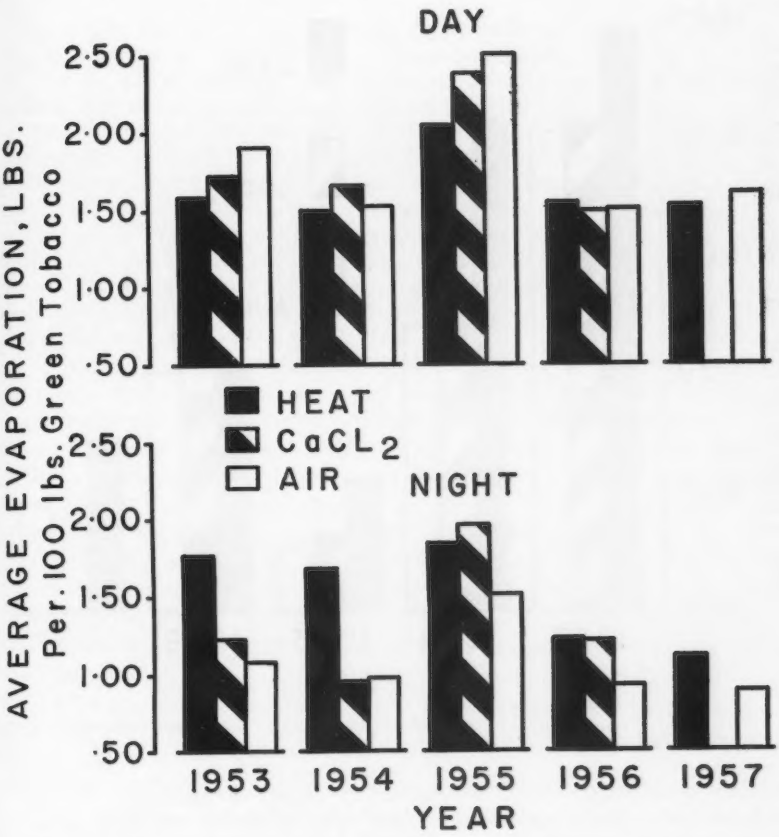

FIGURE 5. Average annual evaporation from burley tobacco by day and night periods for heat, calcium chloride and air curing.

This exact procedure was continued through five curing seasons, 1953 to 1957, except that trials with the calcium chloride dehydrator were discontinued in 1957. To supplement the information obtained in the small pilot barns a similar test was conducted to compare curing in heated and unheated standard type barns of 3-acre capacity in 1956 and 1957.

RESULTS AND DISCUSSION

Figure 2 shows that the mean temperature in the heated barn was consistently higher every year than in the control barn by day as well as by night, although heat was rarely applied during the day and not at all in the daytime during 1955. The daytime difference in temperature between these two barns averaged 4.2° F. Also a slight increase in temperature occurred both by day and by night in the barn containing the calcium

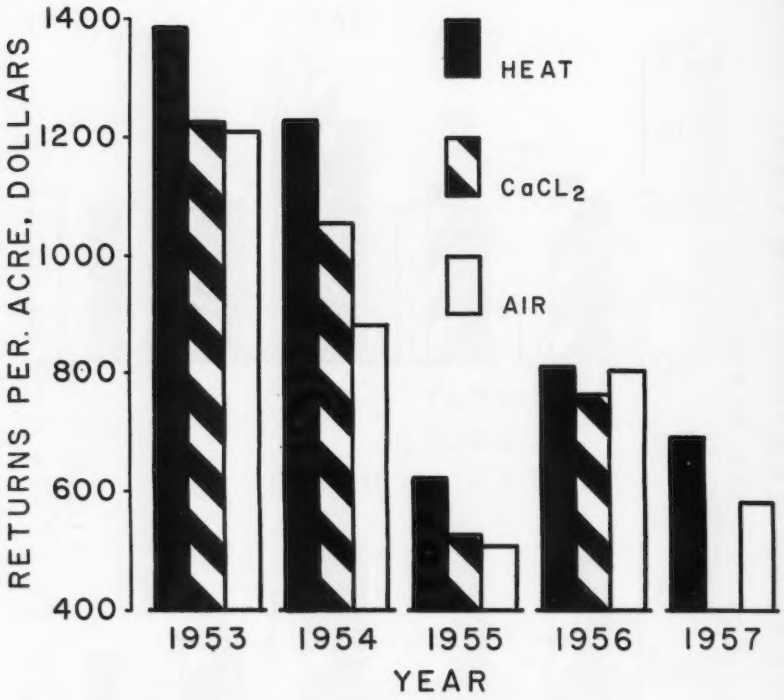

FIGURE 6. Average annual returns per acre from burley tobacco from heat, calcium chloride and air curing.

chloride dehydrator. In either case where higher temperatures occurred without the actual application of heat they probably resulted from a decrease in the amount of heat absorbed by vaporization, owing in turn to the slightly drier condition maintained in the tobacco by the part-time use of either type of drying aid.

In Figure 3 it is shown that adequately regulated heat maintained the mean relative humidity at or near the optimum range during the night while the humidity remained very high, an average of 90 per cent, in the unheated control barn at this time. A more favourable mean relative humidity was maintained in daytime as well by this procedure while the average relative humidity in the air-curing barn was 77 per cent. The calcium chloride dehydrator was less effective either by night or day in this respect.

Figure 4 shows that supplemental heating increased evaporation as measured by Piché evaporimeters particularly at nighttime and to a substantial although lesser extent by day in most years as compared to the unheated control barn. Tobacco in the heated barn exhibited a similar nightly decrease in moisture by weight but in daytime the moisture reduction in this tobacco was inclined to be less than in air-cured tobacco, as

illustrated in Figure 5. This discrepancy between the measured physical drying rate and the loss of moisture from curing tobacco during the day may be accounted for as follows: Some of the moisture transpired from tobacco leaves during curing is translocated from the tobacco stalks which contain a large proportion of the total moisture. Heat curing at night undoubtedly reduced the total moisture in the tobacco more efficiently than air curing. Consequently, less and less moisture remained to be transpired from the leaf surfaces each day than in the air-cured tobacco, resulting in a lower rate of evaporation. Thus the air passing through the heated barn might often be drier during the day than that passing through the air-curing barn, resulting in a greater total evaporation at times from the evaporimeters in the heated barn than in the air-curing barn during this period, e.g., 1954, 1955, 1957.

Figure 6 shows the effect of the three curing methods on dollar returns per acre. Heat curing consistently produced the best results. This method resulted in a 5-cent, and dehydrator curing a 3-cent, higher average price per pound than air curing for the duration of the experiment. (Canadian average price of burley tobacco 1953-1957 was 30.7 cents per pound). The same treatments gave an average increase in yield of 180 pounds and 100 pounds per acre respectively over air curing. The average gain in returns per acre from heat curing for the 5-year period was 159 dollars for an average cost of 36 dollars per acre for propane gas at the rate of 23 cents per gallon. However, the gain in value for tobacco cured with auxiliary heat in 1956 when the weather was exceptionally cold and dry was very little.

When the gas-heating method was tested against natural air curing in standard type, 3-acre curing barns, no appreciable benefit was obtained again in 1956. However, very little gas was used since the tobacco dried sufficiently for the most part without supplementing heat. In 1957, when the weather was exceptionally wet during curing, an increase in returns per acre amounting to 166 dollars was obtained for an expenditure of 30 dollars for propane gas. This gain was accounted for by the combined increase in quality and yield of tobacco.

CONCLUSIONS

1. The optimum average relative humidity of 65 to 70 per cent for curing cigarette burley could be maintained approximately by supplementary heating in air-curing barns when the relative humidity in barns without heat averaged 90 per cent at night and 77 per cent during the day.
2. Supplementary heating substantially improved quality and to some extent increased the yield of cigarette burley tobacco.
3. Dehydration with calcium chloride was much less effective than auxiliary heating in controlling the relative humidity and the quality and yield of burley tobacco.
4. The optimum average relative humidity was obtained with an average temperature increase of 5 to 7° F. above that in unheated air-curing barns.

5. Nighttime evaporation was more than doubled and daytime evaporation increased slightly with the application of auxiliary heat as measured by a Piché evaporimeter.

6. Conversely, evaporation from tobacco tended to decrease during the day and increased during the night only with auxiliary heating.

7. Auxiliary heating increased the average acre value of cigarette burley in a wide variety of curing seasons by 138 dollars at a cost of 32 dollars per acre for propane fuel.

8. Negligible monetary gain was obtained in only one year out of five when the curing season was abnormally cool and dry.

ACKNOWLEDGEMENT

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EFFECTS OF SEED TREATMENTS WITH GIBBERELLIN AND DATES OF SEEDING ON WINTER SURVIVAL AND VEGETATIVE YIELD OF KHARKOV WHEAT

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ABSTRACT

Kharkov winter wheat seeds soaked for 24 hours in 100 p.p.m. gibberellin solution, or in water, were sown on six weekly dates from August 21, to September 25, in the field, in black loam soil, at Edmonton. The chemical stimulated the shoot-growth that occurred before freeze-up but reduced the vigour of subsequent spring-growth, significantly reduced the numbers of survivors from the later seedings, retarded heading, and in most cases significantly reduced the forage yield. The adverse effects of the gibberellin treatments were intensified by the delays in seeding time, which also retarded the development of the untreated wheat.

INTRODUCTION

An earlier paper by this writer has dealt with effects of gibberellin treatments of the foliage upon productivity of Kharkov winter wheat forage, at Edmonton, after being planted in the spring in the field and harvested during summer months of the same year (2). The present report concerns winter-survival, and forage yield of this variety after heading time, following seed treatments with gibberellin prior to planting at various dates in the fall.

MATERIALS AND METHODS

Kernels of the wheat were soaked for 24 hours in aqueous solution of the potassium salt of gibberellic acid containing 100 p.p.m. acid equivalent, before seeding in the field in black loam soil at Edmonton. This concentration of chemical had been shown, in the earlier work, to cause recognizable and not too ephemeral stimulation, without excessive weakening or incipient stunting of the seedlings. Control material was soaked in water. There were 50 viable seeds for each of the treated and control lots, for each of three replicates, at each of six dates of seeding. The wheat was sown 2 inches deep, in 10-foot rows, 2 feet apart. A row of treated seed alternated with a row of check material in each replicate at each date. The six dates of seeding were August 21, 28; September 4, 11, 19, 25, 1957.

Preliminary observations of the emergence of the wheat were made prior to the occurrence of frost and snow on October 3. In 1958, observations were continued to include the numbers of surviving plants (April 25), dates of heading, and yields of vegetation. Commencement of damage by birds necessitated harvesting the plots on July 9, before the wheat matured.

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RESULTS

Visible Effects

Plants of the first four seedings emerged before freeze-up. The gibberellin-treated material exhibited the characteristic increase in leaf and stem length, accompanied by slight chlorosis, as compared with the shorter, more prostrate, darker green controls.

At the time of observations near the end of April, after commencement of the new growing season, the controls had surpassed the treated material in extent of leaf growth, readily apparent upon visual inspection. There was little difference in colour between the plants from treated and untreated seed of the first two seedings, but there were fewer surviving plants from gibberellin-treated seeds. Many of the survivors were weak with considerable reduction in size as compared with the checks. These differences are shown clearly in the photographs taken on June 3, *Plate 1*, Figures 1–6.

Retardation of the treated material became less evident as time passed, but was still shown as a delay in heading in comparison with the respective controls. Again this was progressively more pronounced with the plants of the second to the sixth seeding dates. There was also a definite, although shorter, delay in heading of the control wheat of the later seedings as compared with the check plants of the first group.

Records of Survival, Heading and Forage Yield

The numbers of living plants counted in the spring are shown in Table I as percentages of the original seedings.

Table 1 also summarizes the yields of aerial growth harvested when onslaught by birds threatened to ruin the heads before maturity.

Counts of the numbers of spikes emerged in the various plots at different times during the heading period in June are shown in Table 2.

DISCUSSION AND CONCLUSIONS

This experiment permits of formal statistical comparisons between treated and untreated material only within the dates of seeding. Some

TABLE 1.—PERCENTAGE WINTER SURVIVAL, (APRIL 25), AND OVEN-DRY WEIGHT, (JULY 9), BEFORE MATURITY OF KHARKOV WHEAT FOLLOWING GIBBERELLIN TREATMENT OF SEEDS PLANTED AT DIFFERENT DATES DURING THE PREVIOUS FALL. (MEANS OF 3 REPLICATES)

Date sown		Winter survival		Dry weight	
		Mean (%)	**t value*	*Mean (grams)*	**t value*
Aug. 21	Check	68		1241	
	Treated	56	3.37	1127	0.87
Aug. 28	Check	64		1263	
	Treated	46	1.48	813	6.62
Sept. 4	Check	72		957	
	Treated	51	2.29	592	2.60
Sept. 11	Check	80		1067	
	Treated	48	8.40	620	9.40
Sept. 19	Check	68		801	
	Treated	16	7.07	201	6.54
Sept. 25	Check	83		858	
	Treated	26	70.0	240	12.41

*t value for significance between pairs of treated and untreated plots at 5% level = 4.30, 1% = 9.93.

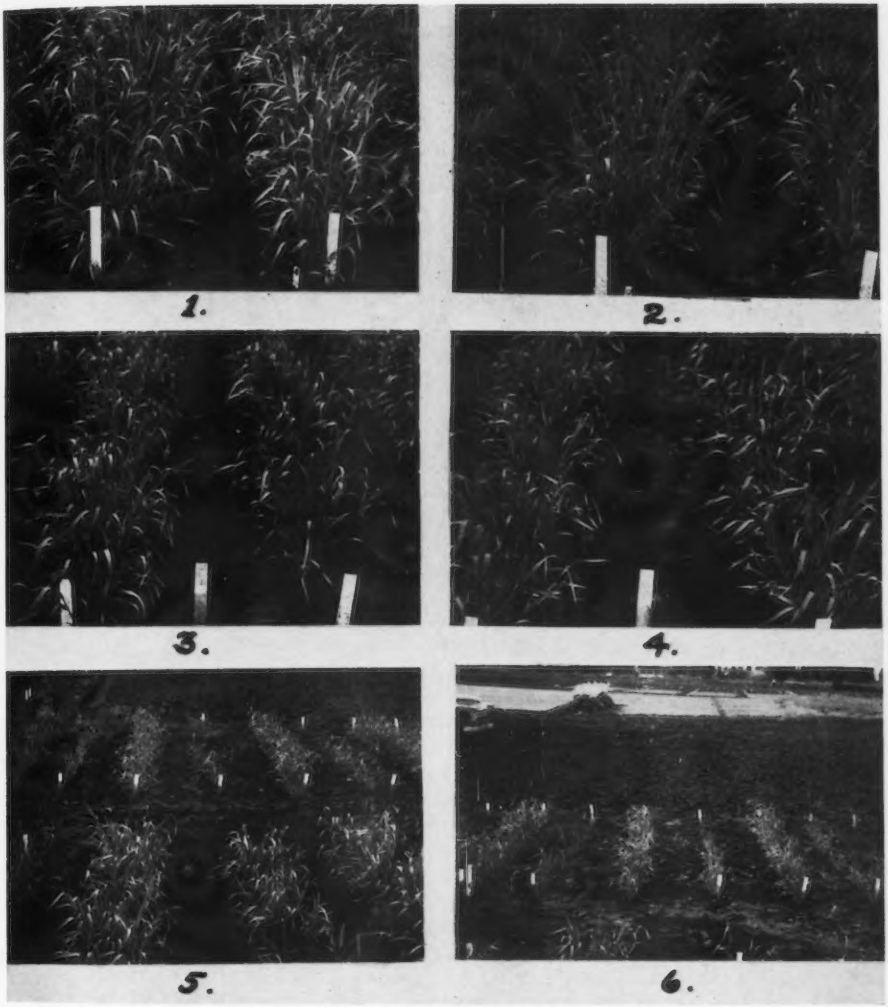

Plate I, FIGURES 1–6. Appearance on June 3, 1958, of Kharkov wheat from untreated and gibberellin-treated seed planted in the field at Edmonton, on August 21, 28; September 4, 11, 19, 25, 1957. Figures 1-6 correspond to the respective seeding times. The gibberellin-affected plants are on the *right* in Figures 1-4 and are also in the alternating rows, with least vegetation, in Figures 5 and 6. The foreground of Figure 5 shows part of the wheat from the third planting, with rows of the fifth seeding date in the background.

TABLE 2.—TOTAL NUMBER OF SPIKES EMERGED IN THREE ROWS OF KHARKOV WHEAT, OBSERVED ON THE DATES SHOWN, FOLLOWING GIBBERELLIN TREATMENT AND DIFFERENT DATES OF SEEDING DURING THE PREVIOUS FALL

Date sown		Count					
		June 10	June 11	June 12	June 13	June 16	June 18
Aug. 21	Check	10	14	51	164	448	—
	Treated	1	3	17	58	162	—
Aug. 28	Check	2	2	9	73	456	—
	Treated	0	0	0	3	118	—
Sept. 4	Check	0	0	1	16	181	—
	Treated	0	0	0	1	90	—
Sept. 11	Check	0	0	0	2	112	—
	Treated	0	0	0	1	31	—
Sept. 19	Check	0	0	0	0	13	105
	Treated	0	0	0	0	1	6
Sept. 25	Check	0	0	0	0	28	133
	Treated	0	0	0	0	1	9

supplementary observations are pertinent. It has been noted that plants from gibberellin-treated seeds were stimulated in their growth rate during the season in which the wheat was planted. Similar response has been reported by Brian *et al.* (1) for wheat seedlings grown in nutrient solutions containing gibberellic acid, and by the current author following seed or foliage treatment of Kharkov wheat (2). In the present work the vigour and the extent of winter survival of the plants from treated seed were decreased. The apparent chemical stimulation of shoot growth in the fall evidently induced a weakened or unhardened condition of the plants, conducive to more winter injury than was shown by the controls. The numerical differences in survival, considered alone, did not reach statistical significance for the first three seedings (Table 1). The negative response to the chemical, however, is emphasized by the obvious qualitative differences between treated and untreated wheat which were progressively more pronounced from the first to the last seeding date (Figures 1-6). It seems clear from the photographs, and the data, that there was an increasing delay in development and an associated decrease in forage yield of both the untreated and treated material paralleling the delay in seeding times (Tables 1 and 2). The reduction in yield associated with delay in planting time, however, was more pronounced with the chemically-treated wheat. The difference in forage yield between treated and untreated wheat was least for the first date of seeding and most for the last two seeding times. The mean yield of the treated material for the initial date of seeding was not significantly less than that of the check. Although it can be seen that this was also true for the September 4th seeding, the result in the latter case seemed to be clearly attributable to the fact that the mean yield of the check plots was lowered by an aberrant 60 per cent reduction in yield of one of the component rows as compared with its two replicates. The difference from the check becomes significant at the 5 per cent level when the figures for only these two replicates are used. The yields from treated wheat of the last two dates of seeding were less than one-third of the

corresponding controls and apparently less than one-quarter of the yield of treated wheat of the first seeding date. On the other hand, the yield of check plants for the final two dates of seeding evidently did not suffer as much, being about two-thirds that of the control for the first date.

This experiment has comprised only one year's results as far as winter survival is concerned. Fortunately, however, for the purpose of this test the winter of 1957-58 was not too severe at Edmonton. Otherwise the total population of treated and untreated plants might have been lost, without showing the effect of the gibberellin. The fact that for none of the six seeding times, before a relatively mild winter, were there any beneficial effects of the chemical treatment, appears to justify the conclusion that extension of work with gibberellin, in this way, is not warranted.

Work in the southern part of Alberta, where the climate is more favourable for production of winter wheat, has shown that early September is the best time for seeding this grain (3). The present results, although obviously in need of additional support, suggest that where there is a cooler fall and shorter frost-free period north of the region established as being fairly reliable for winter wheat, a somewhat earlier date of seeding favours early maturity with perhaps no reduction in yield.

ACKNOWLEDGEMENTS

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RELATION OF SOIL, TEMPERATURE AND TOPOGRAPHY TO FRUIT GROWING IN SUMMERLAND, BRITISH COLUMBIA[1]

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ABSTRACT

The relation of soil class, minimum-temperature differences and topography to the growing of apricot, cherry, peach, apple and pear was studied in an area 7 miles by 4 miles, varying in elevation from 1122 to 1800 feet above m.s.l. The use of an automobile-mounted thermistor thermometer was found useful in establishing minimum-temperature differences. Temperature differences bore a relationship to topography and kinds of fruit being grown satisfactorily. Soil class, except in extreme cases where land was non-arable, bore no relation to the kinds of fruit being grown satisfactorily.

INTRODUCTION

The objectives of this study were: (a) to determine if a consolidated map of a given fruit-growing district could be constructed showing limiting factors such as topography, soil class, temperatures, and other factors with respect to various kinds and varieties of tree fruits; and (b) if such a map were possible to construction, to determine a practical method of preparation. Such map, if expanded to a whole region, should assist industry planners in advising increases or decreases in fruit acreage based on long-range anticipated demands.

To make the study, it was deemed necessary to select a compact district with as wide variation in topography, soil class and temperature as possible and one in which many different kinds and varieties of fruit could be grown satisfactorily. It would also be necessary to construct fruit maps independently of those showing soil class and temperature, if an unbiased relationship between the various factors was to be studied, because the delineation of areas in which various kinds of fruit can be grown, and can be satisfactorily grown, is frequently based only upon observational evidence.

LITERATURE REVIEW

The literature includes many references to the factors under consideration in this study. In general, however, most writers have considered only one, or at the most, two factors at a time. One exception to this is the recent contribution by Mercier and Chapman (25) who studied soil type and air temperature in relation to peach growing in southwest Ontario. In this case, however, the study was not detailed but covered a large area.

Shaw (33) states that the optimum average summer temperatures for apple varieties which are of importance in the Okanagan are: Wealthy 56°F.; Jonathan 59°F., Delicious 59°F.; Newton 60°F.; Stayman 63°F. and Winesap 64°F. No work has been found indicating minimum temperatures

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at which various fruit varieties may be grown satisfactorily, although Brown (10) reports on trunk damage to young trees, Ellison and Close (16) report critical spring minima for various stages of development, and Meader and Blake (24) show that the minimum damaging temperature is in part related to the rate of cooling. Partridge (29) points out that the degree of development of a fruit tree in relation to minimum temperatures is of considerable importance. Mercier and Chapman (25) have selected critical temperatures for peach at Vineland, Ontario, of -20°F. for the dormant wood and −12°F. for dormant blossom buds.

Two methods of determining topoclimate are available (37). The first consists of establishing a network of stationary thermometers (either minimum or recording) and has been used by Lawrence (23), Lamont (22), Albright and Stoker (2), Pomerleau and Ray (32) and others. The second and easier method consists of mounting an electrical thermistor thermometer on an automobile and recording temperatures on selected nights at various stations. This method has been used successfully by Schmidt and Peppler in 1929 [see Geiger (19)], Einarson and Lowe (15), Middleton, Knowles and Spilhaus (26), and Dexter (13). Speed of travel makes little difference to the readings of such thermometers (31).

Geiger (19) presents data from Johnson which show that diurnal fluctuations are greater on clear days than on cloudy days and that minimum temperatures occur just prior to sunrise. These data are supported by illustrations giving the per cent of outgoing radiation with various degrees of cloudiness as follows:

Cloudiness in 10th's	% Outgoing radiation with cloudless sky
0	100
4	80
7	60
9	40
10	20

Mercier and Chapman (25) have recently published a macro-study of peach climates in Ontario. Maps are presented showing isolines of low temperatures, suitable peach soils and a summary climate-soil map for southwestern Ontario.

In non-irrigated areas it is commonly accepted (18) that good orchard soils are deep, sandy to silt loam in texture, somewhat acid in nature, and free from excess moisture. Various minor variations from this ideal have been noted under specific conditions (3, 4, 11, 12, 27, 30). In the irrigated soils the best yields of apple trees have been on deep, heavy soils with good structure and hence with good drainage (35), and on deep silt loam and clay loam soils with good drainage and having a pH between 6.0 and 8.0 (38). Experience in various parts of the world has indicated certain soil preferences for other kinds of deciduous fruit. Deep sandy soils are recommended for peaches (14, 18, 34), while deep well-drained clays are best for pears (5, 18, 21, 28).

The soils of the Okanagan Valley have been surveyed and classified by Kelley and Spilsbury (20). Soil productivity ratings for tree fruits grown in British Columbia have been made (7, 8), considering both soil and climatic factors. These ratings have been further modified (9), based on index ratings proposed by Storie (36) and modified by Freeman (17) and Bowser and Moss (6). This index-rating procedure differs from previous land classifications in that ratings for the various characteristics such as texture, permeability, drainage, alkalinity and slope, instead of being summated, are multiplied to obtain a single index, thus emphasizing a poor characteristic.

MATERIAL AND METHODS

The Municipality of Summerland was selected as being a suitable district within the Okanagan region for this study. It is considered to be near the northern limit within the region for growing apricots, but not too far south to grow McIntosh apples satisfactorily. Its orchards vary in elevation from near lake level (1122 feet) to 1800 feet above sea level, and extend from the Okanagan Lake westward for 4.7 miles. Two valleys run from the main area, one westward, the other northward, in which minimum temperatures were known to differ from those recorded at lake level. The soils of the municipality vary in texture, drainage and fertility. All these variables occur in an area of less than 7 miles north to south and 5 miles east to west.

Throughout this study the following geographic definitions have been used:

Region—a large geographic tract of country (i.e. Okanagan Valley).
District (within a region)—a territory marked off for some purpose. In this case, a municipality or group of municipalities.
Area (within a district)—a piece of land having a distinct topography.
Location (within an area)—a site with relatively narrow boundaries where, in this case, a soil sample or temperature record is taken.

Aerial photographs of the district are available, and were freely used. The only topographical map, however, was at the small scale of 3/4 inch to the mile, with contours at 100-foot intervals. This was transposed to a larger scale (3.82 inches to the mile) and subsequently reduced photographically.

Soil Classes

Although the region has been soil surveyed, this was not done in detail, nor was it done with respect to fruit growing. For this reason a re-survey was made and soils classified for their suitability to fruit growing based on a scheme (8) of rating for the following six factors: texture, salinity, topography, stoniness, erosion and drainage. Each factor was given a per cent rating, multiplied, and reduced to per cent. Soils were then classed on the following basis:

Rating	Description	Class
100–66	"Very good" to "good"	1
65–33	"Fair"	2
32–0	"Poor" to "doubtful"	3

Throughout the district 268 locations were classified. The locations were selected so that all major soil types in the district were represented. No attempt was made to include all soil variations, nor all orchards in the district. At each location a hole was dug to determine soil texture, depth, drainage and erosion. The data (class numbers) were transferred to a large scale map of the district, and areas of similar classes defined. In no case were the area boundaries determined on the site.

Temperature

Temperatures were recorded in degrees F. at 98 locations within the Summerland district on calm, clear fall and winter nights, when the base (Experimental Farm) temperature was estimated to be at a minimum. Calm nights were considered to be those when the mean wind velocity was less than 5 m.p.h. and clear nights those when the cloud cover was no greater than 10 per cent. An automobile-mounted thermistor was used at 3.5 feet above ground level. The instrument was checked with the standard screen and recording thermometers at base prior to and following each run. Time was recorded each 15 minutes on each run in order that necessary adjustments could be made from the recording thermometer at base. Total wind milage at base was recorded for the period of each run.

Temperature differences (location minus base) were determined, adjusting for changes in temperature at base during the run. Temperature differences were averaged for fall (November and December) and winter (January, February and March) runs. The means were then entered on a map and isolines of minimum temperature differences constructed.

The differences in minimum temperature between base and a meteorological station situated in Prairie Valley for the period 1916 to 1922 were determined and compared with those obtained in the present study.

Other Factors

Deficiencies, and in some cases excesses, of various nutrient elements occur throughout the district. Where these may easily be corrected, no account was taken of them. Where corrective methods have not been developed, such as areas of excess alkalinity, soil ratings were reduced.

Kinds of Fruit

All kinds, and most varieties of tree fruits grown in the Okanagan region may be found in the Summerland district. The last fruit tree census (1), taken in 1955, was the basis for the current study. To simplify the study only the following main varieties were considered and grouped as "kind of fruit":

Apricot — Wenatchee Moorpark and Tilton
Cherry — Bing and Lambert
Peach — Veteran, Valiant and Vedette
Pear — Bartlett
Apple — McIntosh, Delicious and its red sports, and Newton.

The distribution of the various kinds of fruit was recorded on a large map indicating the zones in which they *are being grown*. To complete the study, a second map was constructed, showing where the different kinds of fruit are being grown *satisfactorily*. In both cases, boundary-lines between zones were checked in the field.

FIGURE 1. Aerial mosaic of Summerland District. The area within the white boundary is arable. (*Air photos by British Columbia Government*).

It was considered that a fruit is being grown satisfactorily when a planting produced crops frequently enough so that, under favourable marketing conditions, the grower realized a profitable return from his investment. To meet this requirement, apricots, peaches, apples and pears would have to produce a crop 4 years out of 5, while cherries could be profitable with less regular cropping, provided winter tree injury was not excessive.

The opinions of experienced growers within the various zones, and along the lines dividing zones, were considered when deciding where the various kinds of fruit could be grown satisfactorily. Winter injury to trees,

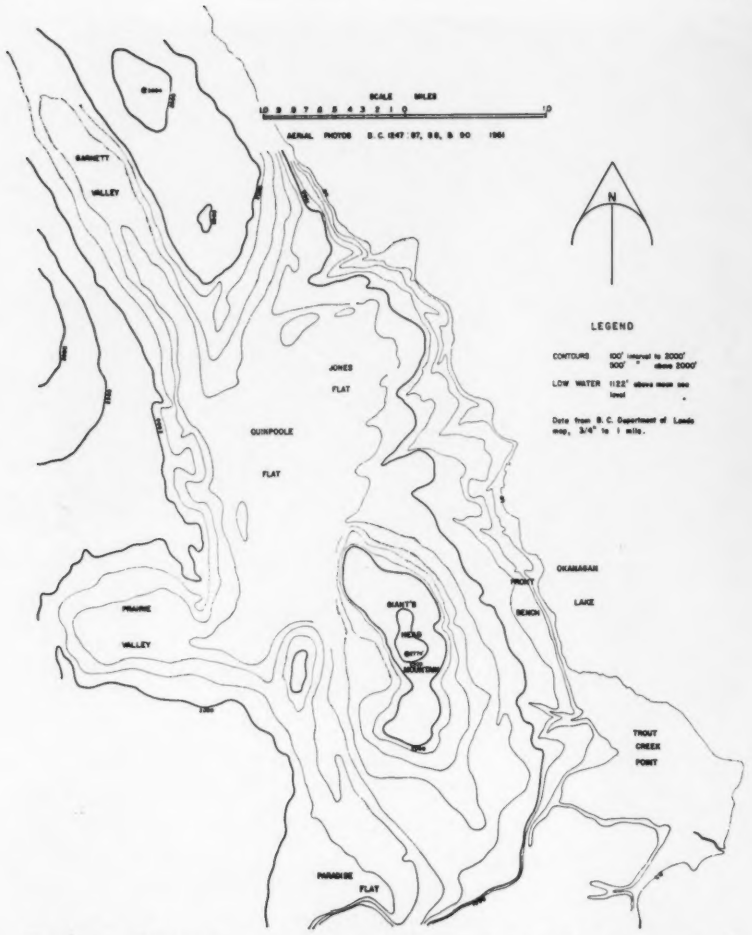

FIGURE 2. Contour map of Summerland District showing the main topographic features and local names.

crop reduction and loss due to spring frosts, reduction of marketable yield due to late fruit maturity, and the general vigour of the trees were the main factors considered.

RESULTS

The Summerland district is bounded on the east by Okanagan Lake and on the west by rock outcrops 2000 feet above lake level. It is divided by Giant's Head Mountain, the peak of which is 1652 feet above lake level. These features are shown in Figures 1 and 2. The main areas within the district are given their local names on Figure 2. Reference will be made to these areas throughout the text.

FIGURE 3. Northeast panorama from Giant's Head Mountain showing the general terrain of the east portion of Jones Flat and the northern portion of the Front Bench. Air flows freely to lake level 300 to 400 feet below.

In general, there is opportunity for the air of all valleys to drain to lake level. This feature is shown in Figure 3. There are, however, small isolated areas throughout the district that are bounded by high land, and are thus potential frost pockets.

Soil

The soils of the district are extremely variable. The sample locations and classes are indicated in Figure 4. Most of the soil is rated as Class 2. The Class 1 soils are found only in small areas along both the north and south Front Bench. Areas of Class 3 soils are scattered throughout the district, the largest of these being at the bottom and north bank of Prairie Valley. In fact, a good portion of this Class 3 area cannot be used for tree-fruit production because of its high alkalinity associated with restricted drainage. This is also true of the Class 3 area in the valley to the west of Giant's Head. The Class 3 areas on Trout Creek Point and to the west of the point are very gravelly, or steep and sandy.

Although class areas have been defined, there are many exceptions. For this reason, individual orchards cannot be assessed on the basis of the present study, unless they were one of the sample locations.

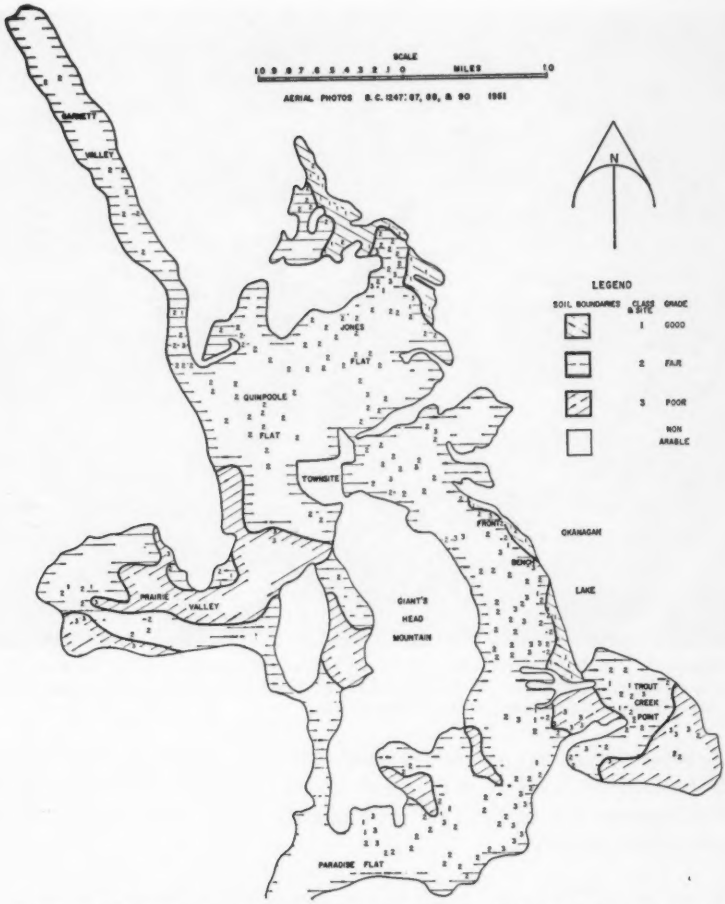

FIGURE 4. Soil class map of Summerland District. Soils were classed for their suitability for fruit growing as follows: Class I, *good;* Class 2, *fair;* Class 3, *poor.* General areas according to class have been determined from sample locations and local knowledge.

Temperature

The winter of 1957-58 was unusually mild and nights largely overcast. A comparison of temperatures recorded during the sample winter with the 42-year mean is shown in Table 1.

Notwithstanding these abnormally high minimum temperatures, six runs (No. 1, 2, 5, 6, 7 and 8) were made on nights when the mean wind velocity was less than 5 m.p.h. and cloudiness was less than 10 per cent. Three runs were made in the fall (No. 1, 2 and 5) and three in the winter (No. 6, 7 and 8). In addition, two runs (No. 3 and 4) in the fall were made when cloud-cover developed shortly after the run had started. These data

TABLE 1.—COMPARISON OF MEAN AND EXTREME FALL AND WINTER TEMPERATURES DURING 42 YEARS AND 1957–58, EXPERIMENTAL FARM, SUMMERLAND, B.C.

Month or period	42-Year Mean		1957–58	
	Mean min.	Extreme daily min.	Mean min.	Extreme daily min.
	°F.	°F.	°F.	°F.
November	36.6	2	30.6	21
December	24.0	–9	29.1	19
Fall mean	30.3		29.9	
January	19.2	–22	28.8	17
February	22.1	–16	33.1	26
March	29.1	3	31.6	23
Winter mean	23.5		31.2	

were discarded. The data for each run are summarized in Table 2. Only one run (No. 7) required adjustment for temperature change at base, and this for only 1 Fahrenheit degree.

The route was changed slightly after the first run, following which all runs were of the same route. The first consisted of 89 locations and all others 98 locations. The following analysis of variance was made, using all acceptable runs:

Source of variance	DF	MS	F		
			Obtained	Required	
				.01	.05
Between seasons	1	114.95	7.50	6.76	3.89
Between locations in seasons	194	15.31	4.95	6.70	3.80
Between runs in locations	383	3.09			

From these results it is apparent that there was a distinct difference between the two seasons with respect to location minus base temperatures. For this reason, the data for each season were mapped individually, averaging three runs for each season. These maps are shown in Figures 5 (Fall) and 6 (Winter).

TABLE 2.—SUMMARY OF DATA FOR TEMPERATURE RUNS IN THE SUMMERLAND DISTRICT

Run	Date	Pacific standard time	Base station data			Range of differences from base temperature	
			Wind	Cloudiness	Temperature	Positive	Negative
No.		from to	M.P.H.	%	°F	°F	°F
1	2/11/57	0430–0700	2.4	0	30	7	7
2	4/11/57	0430–0700	3.0	0	29	7	7
3	16/11/57	0515–0700	2.5	90	34	3	3
4	20/11/57	0615–0745	2.0	100–90	28	4	2
5	10/12/57	0430–0630	3.4	0	29	5	5
6	2/ 1/58	2045–2240	4.5	0	22	5	6
7	10/ 3/58	0500–0645	4.5	0	25–24*	4	6
8	15/ 3/58	0515–0700	4.5	0	25	4	6

*Base temperature dropped from 25°F. to 24°F. at 0530 and remained constant. Temperature differences were adjusted. No adjustment was required for the remaining runs.

TABLE 3.—SUMMARY OF MEAN DIFFERENCES AND MEAN OF SIX GREATEST DIFFERENCES PER MONTH BETWEEN MINIMUM TEMPERATURES RECORDED AT BASE (EXPERIMENTAL FARM) AND PRAIRIE VALLEY, SUMMERLAND METEOROLOGICAL STATIONS OVER A 7-YEAR PERIOD

Item	Mean temperature differences (Prairie Valley—Base) for months in years 1916 to 1922:							
	Oct.	Nov.	Dec.	Mean	Jan.	Feb.	Mar.	Mean
	Fahrenheit Degrees							
Mean min.	−2.2	−2.1	−2.6	−2.3	−3.3	−2.2	−1.8	−2.4
Mean 6 greatest min. differences per month	−4.5	−4.1	−5.0	−4.4	−5.8	−5.3	−4.3	−5.1

A comparison of Figures 5 and 6 clearly shows that the zero temperature difference isoline moves eastward from fall to winter. The warmer areas in the fall (at lake level below the Front Bench) do not show as great temperature difference with base in the winter. Conversely, the colder areas in the fall show a slightly greater temperature difference with base in the winter. There were three small frost-pocket areas evident in the fall that could not be differentiated in the winter. These were in the valleys 0.6 miles north of Quinpoole Flat, 0.6 miles northwest of Paradise Flat, and 0.4 miles northeast of Paradise Flat. There are other small frost pockets in the district which could not be differentiated because of the route taken.

One might question the reliability of such data recorded during an abnormally mild fall and winter. Fortunately a check meteorology station in Prairie Valley (shown at A on Figures 5 and 6) recorded daily maximum and minimum temperatures for a period of 7 years (1916 to 1922) which can be compared with the daily records of base for the same years. A summary of this comparison is shown in Table 3. It will be noted that the check

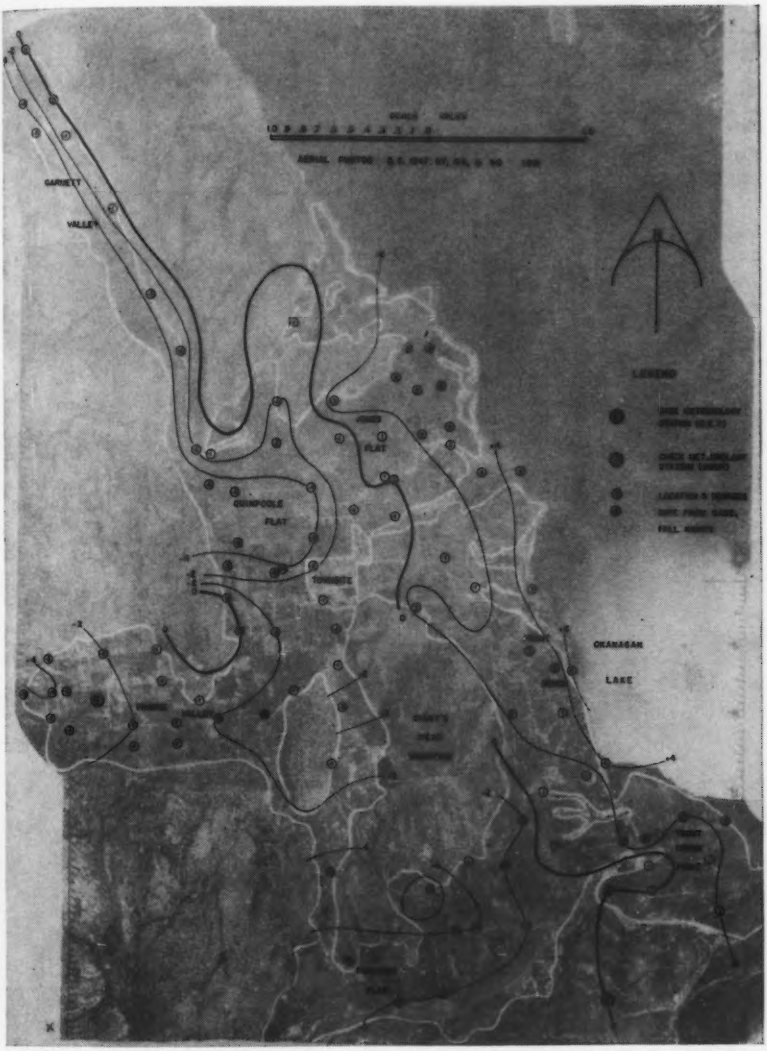

FIGURE 5. Fall night temperature overlay of Summerland District. Isolines indicate mean temperature difference (°F.) from base.

station A is situated between the −2° and −4° isolines on both maps (Figures 5 and 6). From Table 3 it will be seen that the mean difference between daily minimum temperatures are −2.3° and −2.4° for the fall and winter months respectively. This would indicate that the −2° isoline should be moved westward. The isolines were constructed using data obtained on clear, still nights, however, when minimum-temperature differences are

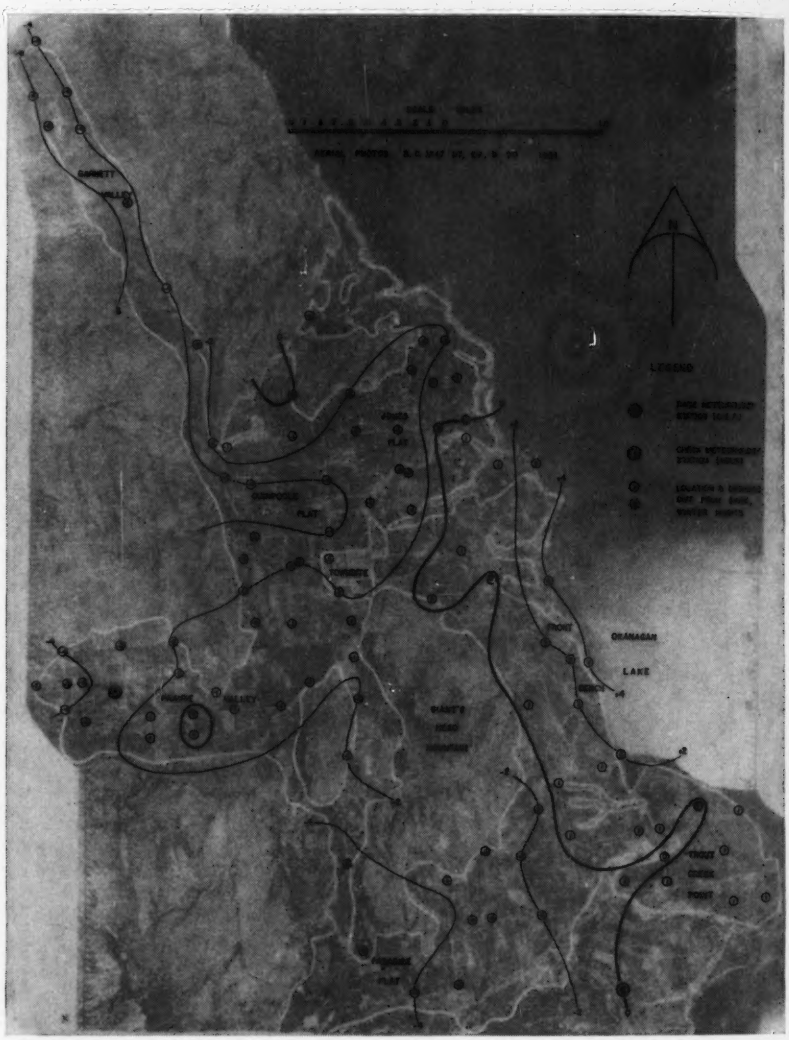

FIGURE 6. Winter night temperature overlay of Summerland District. Isolines indicate mean temperature difference (°F.) from base.

expected to be at a maximum. Considering the mean of the six greatest differences between daily minimum temperatures per month (–4.4° F. for the fall and –5.1° F. for the winter), it would appear that both the –2° and –4° isolines in Prairie Valley should be moved eastward. These data would indicate that greater differences, at least on the negative side, might be expected if the study were over a larger sample of years.

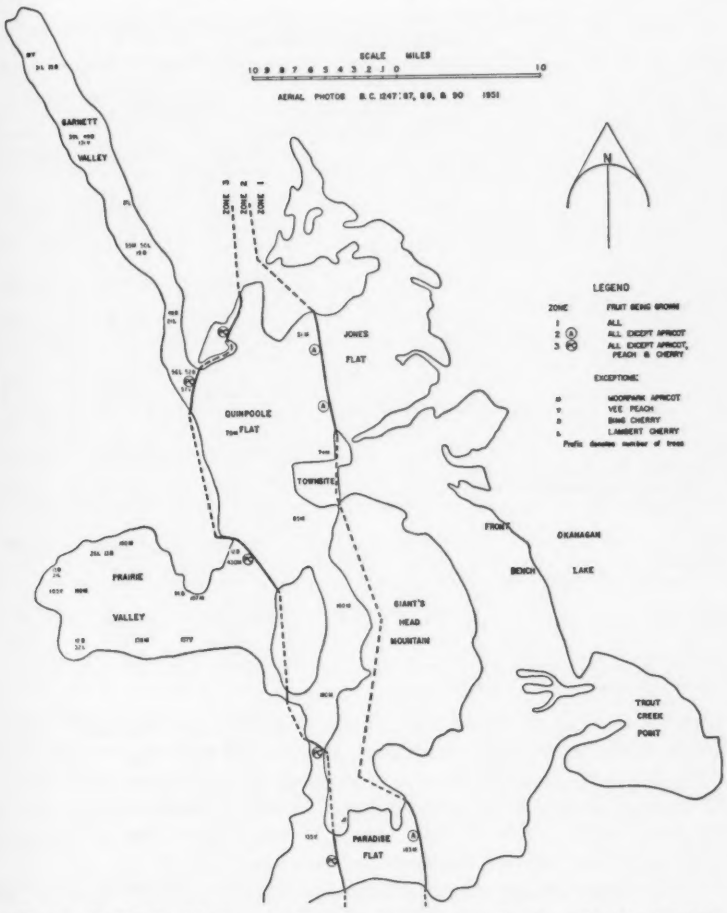

FIGURE 7. Kinds of fruit map of Summerland District showing where fruit is being grown.

Kinds of Fruit

(a) *Where fruit trees are being grown*

The district is divided into three zones which are shown in Figure 7. The zones are "excluding" since they exclude fruits which cannot be grown satisfactorily within their boundaries. All the area between Paradise Flat and Trout Creek Point, Trout Creek Point, the Front Bench (north and south) and most of Jones Flat makes up Zone I. No kinds of fruit are excluded from this zone. Zone II is intermediate and takes in Quinpool Flat, the townsite and east, and the western portion of Paradise Flat. Apricots are excluded, being grown only in isolated locations in this zone, but other kinds of fruit are grown throughout. Zone III extends throughout the

TABLE 4.—DISTRIBUTION OF FRUIT TREES GROWN IN THE SUMMERLAND DISTRICT BY KIND AND ZONE*

Kind	Unit	Total trees by kind	Distribution of fruit trees within zones*		
			Zone 1	Zone II	Zone III
Apricot (Wenatchee and Tilton)	No.	26,924	21,375	3,024	2,525
	%	15.8	79.4	11.2	9.4
Peach (Valiant, Veteran and Vedette)	No.	34,767	26,430	6,776	1,561
	%	20.4	76.0	19.5	4.5
Cherry (Bing and Lambert)	No.	7,391	4,379	2,137	875
	%	4.3	59.2	28.9	11.9
Apple (McIntosh, Red and Common Delicious and Newtown)	No.	59,823	28,376	18,902	12,545
	%	35.0	47.4	31.6	21.0
Pear (Bartlett)	No.	41,842	20,866	8,969	12,007
	%	24.5	49.9	21.3	28.8
Totals	No.	170,747	101,426	39,808	29,513
	%		59.4	22.8	17.8

*See Figure 7 and text for zone boundaries.

western portion of Paradise Flat, Prairie Valley and Garnett Valley. All stone fruits are excluded from Zone III, leaving only apples and pears.

The above zonation is general, and there are many exceptions where small, isolated locations in generally unfavourable zones have been planted to the more tender kinds of fruit. Their locations have been indicated on Figure 7 and their relative numbers are shown in Table 4.

In the Summerland district there is a total of 170,747 trees of the varieties under consideration. The distribution of these trees by zone and kind of fruit is given in Table 4.

Zone I is planted to all kinds of fruit and contains a large portion of the apricots, peaches and cherries, as well as nearly half the apples and pears. In total, the zone contains about 60 per cent of all the Summerland fruit tree population.

Zone II is planted mostly to apples and pears, but also contains a fair portion of the peach and cherry trees. Apricots are grown to a lesser extent in this area and only in isolated locations.

Zone III is planted mainly to apples and pears. Stone fruits are grown only in isolated locations which are considered favourable for various reasons by the growers concerned. Many of these soft fruit plantings were severely injured during the 1955 fall freeze and are being replaced with apples and pears.

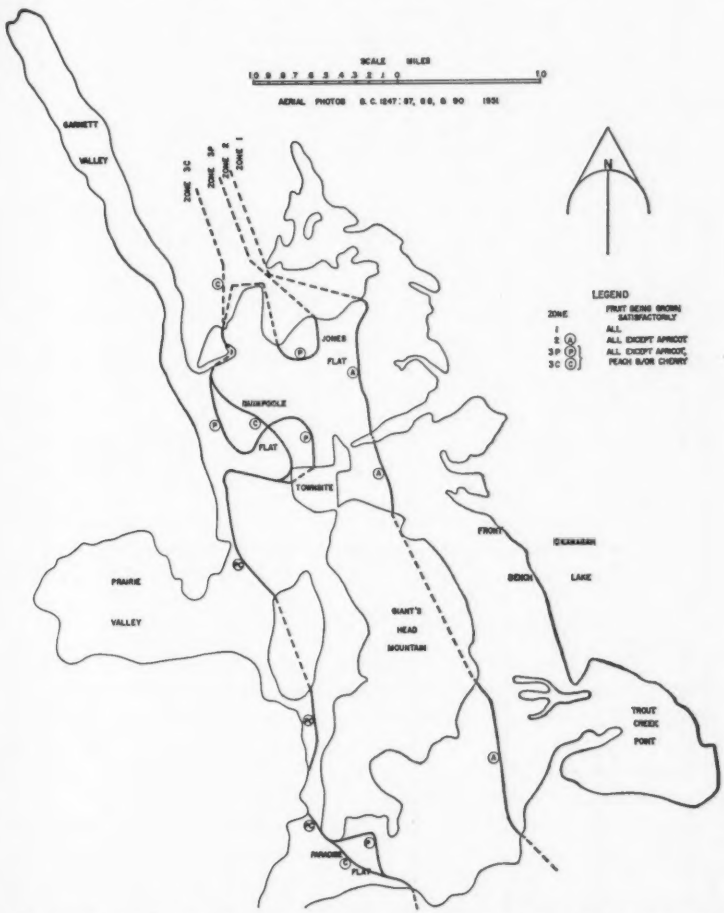

FIGURE 8. Kinds of fruit map of Summerland District showing where fruit is being grown satisfactorily.

(b) *Where fruit trees are being grown satisfactorily*

Figure 8 shows where the various kinds of fruit are growing satisfactorily in Summerland. All kinds of fruit are grown satisfactorily in Zone I; all fruits but apricots can be grown satisfactorily in Zone II; all fruits but apricots and cherries can be grown satisfactorily in Zone III-C; all fruits but apricots and peaches can be grown satisfactorily in Zone III-P; and only apples and pears can be grown satisfactorily in Zone III-CP.

The data from which Figure 8 was constructed are presented in Table 5. Zone II excludes apricots because of spring frosts. Zone III-C excludes cherries due to winter injury. Zone III-P excludes peaches because of late fruit maturity and poor crop quality, thus reducing returns to the grower.

TABLE 5.—DISTRIBUTION OF FRUIT TREES GROWN IN THE SUMMERLAND DISTRICT BY KIND AND ZONE*

Kind of fruit	Unit	Distribution of fruit trees within zones					Total
		Zone I	Zone II	Zone IIIC	Zone IIIP	Zone IIICP	
Apricot (Wenatchee and Tilton)	No.	19,154	5,209	142	318	2,101	26,924
	%	71.1	19.4	.5	1.2	7.8	
Peach (Veteran, Valiant and Vedette)	No.	22,796	9,063	286	946	1,676	34,767
	%	65.6	26.1	.8	2.7	4.8	
Cherry (Bing and Lambert)	No.	3,059	2,533	88	502	1,209	7,391
	%	41.4	34.3	1.2	6.8	16.3	
Apple (McIntosh, Red and Common Delicious, and Newton)	No.	20,053	17,726	906	4,103	17,035	59,823
	%	33.5	29.6	1.5	6.9	28.5	
Pear (Bartlett)	No.	16,473	9,573	478	1,446	13,872	41,842
	%	39.4	22.9	1.1	3.5	33.1	
Total	No.	81,535	44,104	1,900	7,315	35,893	170,747
	%	47.8	25.8	1.1	4.3	21.0	

*See Figure 8 and text for boundaries.

TABLE 6.—TREES GROWN IN ZONE OR ZONES* WHERE SATISFACTORY PRODUCTION IS OBTAINED

Kind of fruit	Zone or zones where kinds of fruit can be grown satisfactorily	Per cent of trees of each kind of fruit grown in proper zone
Apricot (Wenatchee and Tilton)	I	71.1
Peach (Veteran, Valiant, and Vedette)	I, II, IIIc.	92.5
Cherry (Bing and Lambert)	I, II, IIIp	83.4
Apple (McIntosh, Red and Common Delicious and Newton)	I, II, IIIc IIIp, IIIcp	100
Pear (Bartlett)	I, II, IIIc, IIIp IIIcp	100

*See Table 5 for description of zones.

Zones I and II are moved closer to the lake in Figure 8 than in Figure 7, Zones III-C and III-P are additional zones not found in Figures 7, and Zone III-CP corresponds to, but is larger than, Zone III in Figure 7. A large part of Quinpoole Flat was placed in Zone III-CP due to tree winter injury and the frequent occurrence of late spring frosts, which made the production of stone fruits hazardous.

The distribution of fruit trees growing in their correct (satisfactory) zones is shown in Table 6. Two of the five kinds of fruit are extensively planted in unsuitable zones. It is noted that 29 per cent of the apricots are misplanted and 17 per cent of the cherries are planted outside their correct zones. It must be mentioned that Table 6 does not take into consideraion the small, isolated locations which may give satisfactory production of fruits which are generally not suitable for the zone concerned. Conversely, there are similar locations within a zone where the designated fruit will not give satisfactory results.

As previously pointed out, varieties as listed were grouped under kind of fruit. This is a satisfactory system with all fruit except apples, where a marked differential in hardiness is evident. For instance, of the three varieties considered, Newtown is markedly more bud tender than McIntosh or Delicious. For this reason, a separate apple map could be prepared.

DISCUSSION

The outstanding observation from this study is the relationship between where the various kinds of fruit trees can be satisfactorily grown and

the temperature differentials. There appears to be little relationship between soil class and kind of fruit, except where the soil is non-arable or so low in Class 3 that no fruit trees will grow satisfactorily. In the case of temperature differences, however, the zero isotherm (Figure 5) almost coincides with the western boundary of Zone I (Figure 8). Again, the –2° isoline (either fall or winter) nearly corresponds to the location of the western boundary of Zone II. The major exception occurs in the fall where the –2° isoline extends north through Jones Flat and east up Prairie Valley. The winter isoline, however, is very close to the zone boundary.

That there is a relation between minimum temperatures and the kind of fruit which can be grown satisfactorily has been known in general terms. This study has demonstrated the marked effect that small temperature differences have on a mixed tree fruit culture. It may be concluded that a mean difference as little as 4°F. in minimum temperature on clear calm nights determines which kind of fruit can be grown satisfactorily. It may also be concluded that the use of an automobile-mounted thermistor is a suitable method of determining temperature differences between locations and a base station. The base station should be equipped with a recording as well as a standard mercury thermometer. A totalizing anemometer is also a useful tool in determining suitable sample periods.

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THE INHERITANCE OF RUST RESISTANCE

V. THE INHERITANCE OF RESISTANCE TO RACE 15B OF STEM RUST IN TEN VARIETIES OF DURUM WHEAT[1]

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ABSTRACT

The inheritance of resistance to race 15B of stem rust was studied in the varieties St. 464, C.I. 7805, Amarai bianco tipo 142 (P.I. 192179), Arabian, Camadi Abdu tipo 103, Rojal de Almeria (P.I. 191194), C.I. 7870, C.I. 7875, C.I. 8133 and Golden Ball. Rust tests were conducted on F_2 plants from diallel crosses and on F_2 families from back-crosses to the susceptible varieties, Stewart and Nugget. The following genes are present in the varieties:

1. *Srd2* conditioning a type 1-X reaction and present in St. 464, C.I. 7805, P.I. 192179, C.I. 7870, C.I. 7875 and C.I. 8133.
2. *Srd4* conditioning a type 2—2^+ reaction and present in Arabian, P.I. 191194 and Golden Ball.
3. *Srd5* conditioning a type 2 reaction and present in the same varieties as *Srd2*, plus Arabian.
4. *Srd6* conditioning a type 1^- —1 reaction in Camadi.

Two of the genes, *Srd2* and *Srd5*, are additive in effect and together condition a type O; to 1^-reaction.

INTRODUCTION

The appearance and increase in Western Canada of race 15B of wheat stem rust, *Puccinia graminis tritici* Erics and Henn., has made necessary the development of varieties of durum wheat, *Triticum durum* Desf., resistant to this race. Until Ramsey was developed by the North Dakota Agricultural Experiment Station and licensed in Canada in 1956, no variety of durum wheat having resistance to race 15B was available. The heavy losses caused by race 15B in 1953 and 1954 resulted first in a reduction in the acreage of durum wheat in Western Canada, and then in a westward shift in the area of production.

Very few studies on the inheritance of resistance to race 15B in durum wheats have been reported. Heermann (2) and Heermann *et al.* (3) found that C.I. 3255, P.I. 168906 and R. L. 1714 carry a gene, designated *Sr4*, which conditions moderate resistance. Heermann *et al.* also showed that St. 464, P.I. 192179, C.I. 7780, C.I. 7805 and C.I. 8155 each carry two genes, *Sr2* and *Sr5*, which condition an X and a type 1 reaction, respectively, but give a fleck reaction when combined.

In order to develop varieties resistant to race 15B, sources of resistance must be available. Nine varieties of durum wheat showing resistance to race 15B were selected from the International Rust Nursery sent out by the United States Department of Agriculture. In this paper studies on the inheritance of resistance to race 15B are reported for the nine introductions plus the variety Golden Ball.

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[1] This paper is largely taken from a thesis submitted by the senior author to the University of Saskatchewan in partial fulfilment of the requirements for the degree of Master of Science.

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TABLE 1.—THE ORIGIN OF THE PARENT VARIETIES AND THEIR TYPICAL SEEDLING AND ADULT PLANT REACTIONS TO RACE 15B

Name	Accession number	Country of origin	Seedling reaction (pustule type)	Adult reaction (per cent rust)
St. 464	P.I. 191365	Ethiopia	0; −1⁻	0– 1
	C.I. 7805	Ethiopia	0; −1⁻	0– 1
	C.I. 7870	Ethiopia	0; −1⁻	0– 1
	C.I. 7875	Ethiopia	0; −1⁻	0– 1
	C.I. 8133	Ethiopia	0; −1⁻	0– 1
Amarai bianco tipo 142	P.I. 192179	Portugal	0; −1⁻	0– 1
Camadi Abdu tipo 103	P.I. 192168	Portugal	1⁻ – 1	0– 1
Arabian	P.I. 145720	Arabia	2⁻	1– 5
Rojal de Almeria	P.I. 191194	Spain	2	1–10
Golden Ball	C.A.N. 1324	South Africa	2	1–10
Stewart	C.A.N. 3599	North Dakota	4	60–70
Nugget	C.A.N. 3872	North Dakota	4	60–70

MATERIALS AND METHODS

The origin and rust reaction of the ten varieties studied are given in Table 1, along with those of Stewart and Nugget which were used as susceptible parents. To avoid lengthy names in the text, P.I. numbers will be used for two of the varieties (P.I. 192179 and P.I. 191194) and one name will be shortened to Camadi. Seed of the nine varieties in the International Rust Nursery was obtained from W. Q. Loegering of the U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

Diallel crosses were made among the ten rust-resistant varieties. All ten varieties except Golden Ball were crossed and backcrossed to Stewart and five of them were also crossed and backcrossed to Nugget.

The F_2 populations from the crosses were tested primarily for seedling reaction to race 15B although a small number of plants were also tested in a field rust nursery. All of the F_2 families from backcrosses were tested for seedling reaction, and for the cross, Arabian × Nugget[2], a number of F_2 families were also tested in the field.

The methods of conducting rust tests have been described by Knott and Anderson (4).

The seedling reactions were classified according to pustule type, using the system described by Stakman *et al.* (6). On mature plants the per cent of rust infection was read according to the scale outlined by Peterson *et al.* (5).

TABLE 2.—THE RANGE OF SEEDLING REACTIONS IN THE F_2 POPULATIONS FROM THE DIALLEL CROSSES

Variety	St. 406	7805	192179	Arab.	Cam.	191194	7870	7875	8133
C.I. 7805	;–1								
P.I. 192179	;–1	;–1							
Arabian	;–2^+	;–2^-	;–2						
Camadi	;–4	;–4	;–4	;–4					
P.I. 191194	;–4	;–4	;–4	2^-–2^+	$1^=$–4				
C.I. 7870	;–2	;–2	;–2	;–2	;–4	;–4			
C.I. 7875	;–1^+	;–2	;–2^-	;–2	;–4	;–4	;–2^-		
C.I. 8133	;–2	;–2	;–2^-	;–2^+	;–4	;–4	;–2^-	;–2^-	
Golden Ball	;–4	;–4	;–4	2^-–2^+	$1^=$–4	2^-–2^+	;–4	;–4	;–4

The naming of genes for rust resistance in durum wheats is a problem. Ausemus *et al.* (1) recommended the use of the symbol *Sr* plus an arabic numeral to indicate the locus. Heermann *et al.* (3) followed this system but, unfortunately, their symbols duplicate those recommended for common wheat. To avoid this, in the present paper the basic symbol *Srd* (d for durum) is employed instead of *Sr* but the numerals used by Heermann *et al.* are retained. Thus their *Sr2* is the same as S*rd2* in this paper.

RESULTS

A complete summary of the seedling reactions to race 15B of the F_2 populations from the diallel crosses is given in Table 2. The data show whether a cross segregated or not and are used primarily to determine the presence or absence of common genes in the parents.

The actual data obtained from the rust tests are considered either for each variety separately or, in some cases, for a group of similar varieties. The genetic analysis of a variety is based mainly on data from the backcrosses. Additional evidence was obtained from tests on F_2 populations from crosses with Stewart and Nugget. In the seedling tests it was relatively easy to distinguish between plants as susceptible as Nugget and Stewart and those showing at least some degree of resistance. With the field reactions no definite separation into classes was possible. However, plants which carried 50-70 per cent infection were considered to be in the same class as Nugget and Stewart.

St. 464, C.I.7805 and P.I. 192179

The results of rust tests on plants from crosses and backcrosses involving St. 464, C.I. 7805 and P.I. 192179 are given in Tables 3 and 4. Seedling tests using race 15B on the F_2 families from backcrosses involving the three varieties, gave very similar results. In each of the backcrosses to the

TABLE 3.—RESULTS OF SEEDLING TESTS WITH RACE 15B ON F_2 FAMILIES FROM THE BACKCROSSES OF ST. 464, P.I. 192179 AND C.I. 7085 TO STEWART AND NUGGET

Cross	Number of families		Ratio	P
	Segregating	Susceptible		
St. 464 × Stewart²*	66	21	3:1	.50—.95
St. 464 × Nugget²	41	13	3:1	.50—.95
P.I. 192179 × Stewart²	24	5	3:1	.20—.50
C.I. 7805 × Stewart²	27	8	3:1	.20—.50
C.I. 7805 × Nugget²	15	3	3:1	.20—.50

* The superscript indicates the number of crosses made to the recurrent parent.

TABLE 4.—RESULTS OF SEEDLING TESTS WITH RACE 15B ON F_2 POPULATIONS FROM CROSSES INVOLVING ST. 464, P.I. 192179 AND C.I. 7805

Cross	Number of plants		Ratio	P
	Resistant	Susceptible		
St. 464 × Stewart	615	50	15:1	.10—.20
St. 464 × Nugget	319	21	15:1	.50—.95
P.I. 192179 × St. 464	150	0		
C.I. 7805 × St. 464	110	0		
C.I. 7805 × P.I. 192179	91	0		

susceptible parents, Nugget and Stewart, the segregation fits a ratio of three segregating F_2 families:1 susceptible, indicating that resistance is controlled by two independent genes. The F_2 families from each backcross were of four distinct types. In tests where the rust infection was good, some of the families segregated for a type 2 reaction and within these families the segregation of plants fitted a ratio of 3 resistant:1 susceptible. A number of other families segregated in a ratio of 3 resistant:1 susceptible with the reaction of resistant plants ranging from type 1 to type X. Other families segregated for both reactions and in these families a ratio of 15 resistant:1 susceptible plant was obtained. The remainder of the F_2 families were susceptible.

Assuming that 2 independent genes are segregating the four types of families should occur in a ratio of 1:1:1:1. In the backcross, P.I. 129179 × Stewart², a total of 29 families were tested, 8 of which segregated for both types of reaction, 7 segregated for the type X reaction, 9 segregated for the type 2 reaction and 5 were susceptible. This is a satisfactory fit to a 1:1:1:1 ratio. (P. = .50 − .95). Of 35 F_2 families tested from the backcross, C. I. 7805 × Stewart², 11 segregated for both genes, 6 segre-

gated for the type X reaction, 10 segregated for the type 2 reaction and 8 were susceptible. Again the segregation fits a 1:1:1:1 ratio. (P = .50 — .95).

When the backcross, St. 464 × Stewart², was tested, a poor rust infection was obtained on all but one group of 16 families. Of the 16, 5 segregated for both types of reaction, 3 segregated for the type X reaction, 4 segregated for the type 2 reaction and 4 were susceptible. The segregation is a satisfactory fit to a 1:1:1:1 ratio. (P. = .50 — .95). In the remaining families it was possible to determine which families were segregating but the separation into the four types was not distinct. Similar difficulties were encountered in the backcrosses, St. 464 × Nugget² and C.I. 7805 × Nugget².

The data from the tests on F_2 plants of the crosses, St. 464 × Stewart and St. 464 × Nugget, corroborate the backcross results. In seedling tests, the segregation of F_2 plants fits a ratio of 15 resistant:1 susceptible.

The diallel crosses, C.I. 7805 × St. 464, P.I. 192179 × St. 464 and C.I. 7805 × P.I. 192179, were tested for seedling reaction to race 15B. All the F_2 plants from each cross were as resistant as the parents. Although the numbers are small, the fact that no plants were more susceptible than the parents indicates that the two genes for resistance in each variety are identical.

The 2 genes of the varieties, St. 464, C.I. 7805 and P.I. 192179, are independent and have an additive effect on resistance to race 15B. The combination of the 2 genes results in high resistance characterized by hypersensitive flecking.

The conclusion that St. 464, P.I. 192179 and C.I. 7805 have 2 common genes for resistance to race 15B agrees with the report by Heermann *et al.* (3). The gene conditioning a type 1-X reaction will be called *Srd2* and the one conditioning a type 2 reaction, *Srd5*.

Arabian

The data obtained from the crosses and backcrosses involving Arabian are given in Tables 5 and 6. The results from seedling tests on the backcrosses, Arabian × Stewart², and Arabian × Nugget², are conflicting.

TABLE 5.—RESULTS OF TESTS WITH RACE 15B ON F_2 FAMILIES FROM BACKCROSSES OF ARABIAN TO NUGGET AND STEWART

Cross and type of test	Number of families		Ratio	P
	Segregating	Susceptible		
Seedling tests				
Arabian × Stewart²	84	6	7:1 15:1	.05—.10 .95—.99
Arabian × Nugget²	98	31	3:1	.50—.95
Field tests				
Arabian × Nugget²	23	8	3:1	.50—.95

The observed segregation in the backcross to Stewart will fit both a 7:1 and a 15:1 ratio, suggesting that either 3 or 4 genes are involved in resistance. On the other hand, the results from the backcross to Nugget are a good fit to a 3:1 ratio, indicating that Arabian carries only 2 genes. Field tests on a portion of the Nugget backcrosses agreed with the seedling tests. Unfortunately, due to a shortage of seed, the backcross families could not be retested.

TABLE 6.—RESULTS OF RUST TESTS ON F_2 POPULATIONS FROM THE CROSSES INVOLVING ARABIAN

Cross and type of test	Number of plants		Ratio	P
	Resistant	Susceptible		
Seedling tests				
Arabian × Stewart	289	21	15:1	.50—.95
Arabian × Nugget	282	19	15:1	.95—.99
Arabian × St. 464	517	0	—	
Arabian × P.I. 192179	82	0	—	
Arabian × C.I. 7805	84	0	—	
Field tests				
Arabian × St. 464	349*	0	—	

* Plants showed up to 50% infection (3 plants in 1 family) but were not considered to be as susceptible as Stewart and Nugget.

TABLE 7.—RESULTS OF RUST TESTS ON F_2 POPULATIONS FROM CROSSES OF SINGLE ARABIAN PLANTS WITH BOTH NUGGET AND STEWART

Cross	Number of plants		Ratio	P
	Resistant	Susceptible		
Nugget × Arabian (1)*	72	7	15:1	.20—.50
Stewart × Arabian (1)	66	6	15:1	.20—.50
Nugget × Arabian (2)	64	8	15:1	.05—.10
Stewart × Arabian (2)	72	5	15:1	.50—.95
Nugget × Arabian (3)	35	3	15:1	.50—.95
Stewart × Arabian (3)	62	6	15:1	.20—.50
Nugget × Arabian (4)	75	7	15:1	.20—.50
Stewart × Arabian (4)	34	4	15:1	.20—.50

* The number designates the Arabian plant used as a male parent.

The hypothesis that Arabian has only 2 genes for resistance was supported by results obtained when F_2 plants of the crosses, Arabian × Stewart and Arabian × Nugget were tested for seedling and adult reaction to race 15B. In both crosses the segregation of F_2 plants fits a ratio of 15 resistant:1 susceptible.

The possibility that Arabian behaved differently in crosses with Nugget and Stewart, was tested further. Four plants of Arabian were selected and each was used as the pollen parent in crosses with both Nugget and Stewart. The 8 F_2 populations were then tested with race 15B. The results are given in Table 7. The segregations were similar in all the crosses and fitted a 15:1 ratio, although there is a tendency for an excess of susceptible plants. The anomalous results obtained in the Stewart backcross can be explained by assuming that the original seed stock was not pure. It seems probable that Arabian has only 2 genes for resistance.

The 2 genes in Arabian both condition a type 2 reaction with one possibly giving a slightly poorer resistance than the other. Together they condition a pustule that is about a type 1 in size but has the characteristic "green islands" of type 2.

The data from the cross, Arabian × St. 464, suggest the presence of a common gene in the two varieties. Of 517 F_2 plants classified for seedling reaction to race 15B, none was susceptible. The reaction of resistant plants ranged from fleck to type 2. In field tests, no completely susceptible plants were obtained when 349 F_2 plants were tested for adult plant reaction. Similar results were obtained in the crosses Arabian × P.I. 192179 and

TABLE 8.—RESULTS OF SEEDLING TESTS WITH RACE 15B ON F_2 FAMILIES FROM BACKCROSSES OF CAMADI TO NUGGET AND STEWART

Cross	Number of families		Ratio	P
	Segregating	Susceptible		
Camadi × Stewart[2]	41	42	1:1	1.0
Camadi × Nugget[2]	13	15	1:1	.50—.95

TABLE 9.—RESULTS OF SEEDLING RUST TESTS ON F_2 POPULATIONS FROM CROSSES INVOLVING CAMADI

Cross	Number of plants		Ratio	P
	Resistant	Susceptible		
Camadi × Arabian	575	3	63:1	.05—.10
Camadi × St. 464	272	6	63:1	.20—.50
Camadi × P.I. 192179	88	1	63:1	1.0
Camadi × C.I. 7805	75	1	63:1	1.0

Arabian × C.I. 7805. The F_2 plants of these crosses, when tested for seedling reaction, were either very resistant or moderately resistant to race 15B. The absence of fully susceptible plants in each of the crosses clearly indicates that the varieties have one gene in common. Since a gene which conditions a type 2 reaction, *Srd5*, has been identified in the varieties St. 464, C.I. 7805 and P.I. 192179 and a gene conditioning a similar reaction has been identified in Arabian, it is logical to conclude that the gene is common to all four varieties.

TABLE 10.—RESULTS OF SEEDLING TESTS WITH RACE 15B ON F_2 FAMILIES FROM BACKCROSSES OF P.I. 191194 TO STEWART

Cross	Number of families		Ratio	P
	Segregating	Susceptible		
P.I. 191194 × Stewart[2]	18	16	1:1	.50—.95

TABLE 11.—RESULTS FROM SEEDLING TESTS ON F_2 POPULATIONS FROM THE CROSSES INVOLVING P.I. 191194

Cross	Number of plants		Ratio	P
	Resistant	Susceptible		
P.I. 191194 × St. 464	158	5	63:1	.10—.20
P.I. 191194 × P.I. 192179	81	2	63:1	.50—.95
P.I. 191194 × C.I. 7805	62	2	63:1	.20—.50
P.I. 191194 × Arabian	326	0	—	—
P.I. 191194 × Camadi	64	7	15:1	.20—.50

TABLE 12.—RESULTS OF SEEDLING TESTS WITH RACE 15B ON F_2 FAMILIES FROM THE BACKCROSSES OF C.I. 7870, C.I. 7875 AND C.I. 8133 TO STEWART AND NUGGET

Cross		Number of families		Ratio	P
		Segregating	Susceptible		
C.I. 7870 × Stewart[2]	A	24	26	1:1	.50—.95
	B	35	9	3:1	.20—.50
C.I. 7875 × Stewart[2]	A	11	17	1:1	.20—.50
	B	38	16	3:1	.20—.50
C.I. 8133 × Stewart[2]	A	26	28	1:1	.50—.95
	B	54	18	3:1	1.0
C.I. 8133 × Nugget[2]		22	5	3:1	.20—.50

The second gene which conditions a type 2 — 2^+ reaction in Arabian will be designated *Srd4*, since data given in the section on Golden Ball suggest that it is identical to a gene in the latter variety which was labelled *Sr4* by Heermann *et al.*

Camadi

The results of rust tests on F_2 plants from the crosses and backcrosses involving Camadi are given in Tables 8 and 9.

When tested with race 15B in the seedling stage, the F_2 families from the backcrosses, Camadi × Stewart[2] and Camadi × Nugget[2], give a good fit to a ratio of 1 segregating:1 susceptible. Within the segregating families, a ratio of 3 resistant:1 susceptible F_2 plant was obtained. The reaction of resistant plants ranged from type 1- to as high as type X^+. The results indicate that Camadi has a single, incompletely dominant gene for resistance to race 15B. The typical reaction of a seedling homozygous for the gene was type 1^- to 1 but heterozygotes ranged from type 2 to X^+.

The F_2 plants from the crosses, Camadi × Arabian, Camadi × P.I. 192179, Camadi × C.I. 7805 and Camadi × St. 464 segregated for resistance, indicating that the varieties do not have a gene in common for resistance to race 15B. The segregations obtained from the three crosses fit the ratio of 63 resistant plants:1 susceptible expected on the assumption that C.I. 7805, P.I. 192179 and St. 464 have 2 genes and Camadi 1 gene for resistance to race 15B.

Apparently the Camadi gene is independent of the genes so far mentioned and it is, therefore, designated as *Srd6*.

P.I. 191194

The results from crosses and backcrosses involving P.I. 191194 are given in Tables 10 and 11.

In seedling tests, the F_2 families from the backcross, P.I. 191194 × Stewart[2], gave a good fit to a ratio of 1 segregating:1 susceptible. Within segregating families a ratio of 3 moderately resistant plants (type 2 — 2^+): 1 susceptible plant (type 4) was obtained. The results show that P.I. 191194 has a single dominant gene governing a type 2 — 2^+ reaction to race 15B.

The cross, P.I. 191194 × Arabian, did not segregate for resistance to race 15B, indicating that the varieties have a gene in common. All plants were moderately resistant, ranging from a type 2^- to a type 2 reaction. Apparently Arabian and P.I. 191194 have either *Srd4* or *Srd5* in common. The crosses of P.I. 191194 with St. 464, C.I. 7805 and P.I. 192179, all of which carry *Srd5*, segregated for resistance. It is evident, therefore, that P.I. 191194 does not have *Srd5* but must have *Srd4*. The segregation of the F_2 plants was a good fit to a ratio of 63 resistant:1 susceptible indicating that 3 genes were involved, 2 from St. 464, C.I. 7805 and P.I. 192179 respectively, and 1 from P.I. 191194.

The moderate resistance of P.I. 191194 to race 15B is, therefore, controlled by a single dominant gene, *Srd4*.

TABLE 13.—RESULTS OF SEEDLING TESTS WITH RACE 15B ON F_2 POPULATIONS FROM THE CROSSES INVOLVING C.I. 7875, C.I. 7870 AND C.I. 8133

Cross	Number of plants		Ratio	P
	Resistant	Susceptible		
C.I. 7875 × C.I. 8133	245	0		
C.I. 7870 × C.I. 7875	259	0		
C.I. 7870 × C.I. 8133	236	0		
C.I. 7875 × St. 464	323	0		
C.I. 7870 × St. 464	307	0		
C.I. 8133 × St. 464	323	0		
C.I. 7875 × P.I. 192179	168	0		
C.I. 7870 × P.I. 192179	101	0		
C.I. 8133 × P.I. 192179	132	0		
C.I. 7875 × C.I. 7805	70	0		
C.I. 7870 × C.I. 7805	51	0		
C.I. 8133 × C.I. 7805	106	0		
C.I. 7875 × Arabian	282	0		
C.I. 7870 × Arabian	309	0		
C.I. 8133 × Arabian	267	0		
C.I. 7875 × P.I. 191194	172	3	63:1	1.0
C.I. 7870 × P.I. 191194	205	6	63:1	.20—.30
C.I. 8133 × P.I. 191194	225	4	63:1	1.0
C.I. 7875 × Camadi	128	3	63:1	.30—.50
C.I. 7870 × Camadi	138	1	63:1	.30—.50
C.I. 8133 × Camadi	114	2	63:1	1.0

C.I. 7870, C.I. 7875 and C.I. 8133

The results of rust tests on the F_2 families from backcrosses involving the varieties, C.I. 7870, C.I. 7875 and C.I. 8133, are given in Table 12. The backcross data proved to be rather confusing. For all three varieties the ratio of segregating to susceptible families fell between 1:1 and 3:1, and, moreover, the results were heterogeneous. When the backcross families were traced back to their parents it appeared that in some cases the resistant parent had been homozygous for one gene and in other cases for two. In addition, the results suggested that a few plants had been homozygous for one gene but heterozygous for a second. Unfortunately, in order to sample as many F_1 plants as possible, only one head on each

was backcrossed. Thus the number of backcross families derived from any one F_1 plant was small and in many cases the segregation obtained would fit either a 1:1 or 3:1 ratio. In Table 12 the results from each backcross have been grouped into two classes, A and B, depending on whether they are closest to a 1:1 or a 3:1 ratio. It is concluded that all plants in the three varieties are homozygous for one gene for resistance but may be homozygous resistant, segregating, or homozygous susceptible at a second locus.

The results of tests on F_2 populations from crosses involving C.I. 7875, C.I. 7870 and C.I. 8133 are given in Table 13. All of the F_2 plants from crosses between the three varieties were resistant with reactions ranging from fleck to type 2^-. In many F_2 families all of the plants were as resistant as the parents, that is they gave a fleck to type 1 reaction It is evident that the varieties have at least one gene for resistance to race 15B in common. Furthermore, the fact that in certain families all of the plants were highly resistant suggests that the parents in these cases had two genes in common.

Results very similar to the above were obtained in the crosses of C.I. 7875, C.I. 7870 and C.I. 8133 with St. 464, P.I. 192179 and C.I. 7805. Again the most susceptible F_2 plants gave a 2^- reaction and in many families all of the seedlings were as resistant as the parents. It was concluded, therefore, that in C.I. 7870, C.I. 7875 and C.I. 8133, the plants that were homozygous for two genes, carried the same two genes as St. 464, P.I. 192179 and C.I. 7805, that is *Srd2* and *Srd5*.

Further evidence comes from the crosses with Arabian which gives a type 2 seedling reaction. In each of the three crosses, no plant gave a reaction more susceptible than type 2. Since Arabian carries *Srd5* in

TABLE 14.—RESULTS OF SEEDLING TESTS ON F_2 POPULATIONS FROM CROSSES INVOLVING GOLDEN BALL

Cross	Number of plants		Ratio	P
	Resistant	Susceptible		
Golden Ball × Arabian	220	0		
Golden Ball × P.I. 191194	162	0		
Golden Ball × Camadi	75	3	15:1	.20—.50
Golden Ball × St. 464	91	1	63:1	1.0
Golden Ball × C.I. 7805	17	1	63:1	.50—.95
Golden Ball × P.I. 192179	57	1	63:1	1.0
Golden Ball × C.I. 7870	147	5	*	
Golden Ball × C.I. 7875	100	2	*	
Golden Ball × C.I. 8133	114	4	*	

* These ratios fall between 15:1 and 63:1 (see text).

TABLE 15—GENE EXPRESSION AND THE RUST REACTION OF HOMOZYGOUS PLANTS

Gene	Gene expression	Reaction of homozygotes	
		Seedlings	Mature plants
Srd2	Dominant	1–X	R
Srd4	Dominant	2	MR
Srd5	Dominant	2	MR
Srd6	Partially dominant	1⁻–1	R

TABLE 16.—PROBABLE GENOTYPE OF EACH VARIETY

Variety	Genotype			
St. 464	*Srd2 Srd2*		*Srd5 Srd5*	
C.I. 7805	*Srd2 Srd2*		*Srd5 Srd5*	
P.I. 192179	*Srd2 Srd2*		*Srd5 Srd5*	
C.I. 7870	*Srd2 Srd2*		*Srd5 Srd5*	
C.I. 7875	*Srd2 Srd2*		*Srd5 Srd5*	
C.I. 8133	*Srd2 Srd2*		*Srd5 Srd5*	
Arabian		*Srd4 Srd4*	*Srd5 Srd5*	
P.I. 191194		*Srd4 Srd4*		
Golden Ball		*Srd4 Srd4*		
Camadi				*Srd6 Srd6*

common with St. 464, P.I. 192179 and C.I. 7805, it is evident that the same gene is present in C.I. 7870, C.I. 7875 and C.I. 8133. If, however, the latter three carried only *Srd5* in common with the former three, all crosses between a variety in one group and a variety in the other group should segregate a considerable number of plants giving a type 2 reaction. Such was not the case.

The crosses of C.I. 7870, C.I. 7875 and C.I. 8133 with P.I. 191194 and Camadi segregated for resistance, indicating that the parents do not have a gene for resistance in common. The segregations fit a 63:1 ratio.

Golden Ball

Golden Ball is moderately resistant to race 15B and gives a type 2—2⁺ reaction in the seedling stage and from 5—20 per cent infection in the adult stage. It was crossed with all nine of the 15B-resistant varieties to test for the presence of common genes. The results of seedling tests on the F_2 populations are given in Table 14. In two of the crosses, Golden Ball × Arabian and Golden Ball × P.I. 191194, all of the F_2 plants gave a type 2⁻ to 2⁺ reaction. Since P.I. 191194 carries only gene *Srd4* and this gene is also present in Arabian, it is evident that Golden Ball has *Srd4*.

Unfortunately, Golden Ball was not crossed with a susceptible variety and, consequently, the number of genes it carries can be deduced only from segregations in crosses with resistant parents. The cross with Camadi, which has only 1 gene for resistance, gave a segregation which will fit a 15:1 or 2-gene ratio. Since 1 gene came from Camadi, only 1 can have

come from Golden Ball. In the crosses with St. 464, C.I. 7805, and P.I. 192179, all of which carry 2 genes for resistance, the segregations will fit a 63:1 ratio. This agrees with the hypothesis that Golden Ball has only 1 gene.

The crosses of Golden Ball with C.I. 7870, C.I. 7875 and C.I. 8133 segregated for resistance and the segregations fall between a 15:1 and 63:1 ratio. Again the results suggest that the latter three varieties may carry either one or two genes.

DISCUSSION AND CONCLUSIONS

In a breeding program to produce a high quality, rust resistant durum variety, a knowledge of the inheritance of rust resistance in potential parents is very useful. The results reported in this paper show that a number of genes providing satisfactory resistance to race 15B are readily available. The genes are described in Table 15.

The ten varieties studied fall roughly into three groups as is shown in Table 16. The first group contains six varieties, five from Ethiopia, St. 464, C.I. 7805, C.I. 7870, C.I. 7875 and C.I. 8133, and one from Portugal, P.I. 192179. All six carry 2 genes, *Srd2* and *Srd5*, which together condition a very high degree of resistance to race 15B. The second group contains three varieties, Arabian, P.I. 191194 and Golden Ball, all of which carry *Srd4*, a gene for moderate resistance. Arabian also carries *Srd5*. Camadi differs from the rest of the varieties in carrying a single gene, *Srd6*, which conditions good resistance to race 15B.

The data obtained in this study agree with those reported by Heermann *et al.* (3) wherever the same varieties were studied.

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INHERITANCE OF SHADES OF BRONZE AND PINK FLOWERS OF *ANTIRRHINUM MAJUS*[1]

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ABSTRACT

The influence of three genes, *Inc*, *Pal* and *Dil*, on the intensity of anthocyanin pigmentation in bronze and pink flowers of *Antirrhinum majus* was investigated. Formation of anthocyanin requires the presence of one allele each of *Inc* and *Pal*. Flowers of both pal^{rec} pal^{rec} and pal^{tub} pal^{tub} plants are acyanic (with *eos eos*) like those of *inc inc* plants. *Inc* and *Pal* are incompletely dominant. *Dil* is a dominant intensifier. The dosage effects of the three genes are additive, resulting in four shades of bronze and pink. The effect of one *Inc* allele in increasing pigmentation intensity approximates that of one *Pal* allele or that of one or two *Dil* alleles.

Genetic evidence on gene synonymy shows that the *p* of Geissman and co-workers, Haney's *iv* and the authors' *inc* are identical. The allelic genes *S* and *d* of Wheldale are members of the *Pal* series and are not equivalent to the *p* of Geissman and co-workers.

Flower colour genotypes of 13 commercial varieties of snapdragons are reported.

INTRODUCTION

The principal flower colours of the snapdragon (*Antirrhinum majus*) are white, yellow, ivory, bronze, pink, crimson and magenta. This investigation is mainly concerned with the interactions of three genes that control the intensity of anthocyanin pigmentation in bronze and pink flowers. Initially, however, it was necessary to determine whether the genes in our material were the same as those reported by other workers. The flower colour genotypes of 13 horticultural varieties were established in the course of this preliminary work.

Inheritance of the Principal Flower Colours

There is general agreement that four genes control the inheritance of the principal flower colour classes of *A. majus* (2, 4, 5, 7, 13). Four pigments—ivory apigenin, yellow aureusidin, pink pelargonidin and magenta cyanidin (all present as glycosides)—appear to be the most important in determining these colour phenotypes (3, 4, 5, 6). Table 1 shows the genotypes and pigment composition of the seven flower colour classes.

Niv is the basic factor for all pigment formation; flowers of *niv niv* plants are dead white. All *Niv* plants have apigenin throughout the corolla and aureusidin in the corolla lips. In *Sulf* individuals the yellow aureusidin is restricted to the palate spot of the lower lip whereas it extends throughout the lips of *sulf sulf* plants. *Inc* controls anthocyanin formation throughout the corolla. In *eos eos* plants the anthocyanidin is pelargonidin, whereas in *Eos* plants it is cyanidin. Bronze results from a mixture of aureusidin and pelargonidin. Crimson is a mixture of aureusidin and cyanidin. Because aureusidin does not form in the corolla tube, the tubes of bronze flowers are pink, and the tubes of crimson flowers are magenta.

[1] Contribution No. 956, Horticulture Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.

TABLE 1.—THE PRINCIPAL FLOWER COLOUR CLASSES OF *A. majus*, THEIR GENOTYPES AND IMPORTANT PIGMENTS

Phenotypes	Necessary genotype								Pigment aglycones		
									Flavone	Aurone	Anthocyanidins
white	*niv*	*niv*	—	—	—	—	—	—	—	—	—
yellow	*Niv*	—	*sulf*	*sulf*	*inc*	*inc*	—	—	Apigenin	Aureusidin (lips)	—
ivory	*Niv*	—	*Sulf*	—	*inc*	*inc*	—	—	Apigenin	Aureusidin (palate)	—
bronze	*Niv*	—	*sulf*	*sulf*	*Inc*	—	*eos*	*eos*	Apigenin	Aureusidin (lips)	Pelargonidin
pink	*Niv*	—	*Sulf*	—	*Inc*	—	*eos*	*eos*	Apigenin	Aureusidin (palate)	Pelargonidin
crimson	*Niv*	—	*sulf*	*sulf*	*Inc*	—	*Eos*	—	Apigenin	Aureusidin (lips)	Cyanidin
magenta	*Niv*	—	*Sulf*	—	*Inc*	—	*Eos*	—	Apigenin	Aureusidin (palate)	Cyanidin

TABLE 4.—PHENOTYPES AND GENOTYPES OF THE PARENT VARIETIES, AND PHENOTYPES APPEARING IN THE F_1'S

	Francis Schlegel	Sutton's Intermediate Yellow	Y-46	Margaret	Y-19B	Y-19P
Francis Schlegel (yellow) *NN ss ii PP EE*	Yellow	Yellow	Crimson	Ivory	Crimson	Crimson, Magenta
Sutton's Intermediate Yellow *NN ss ii p^t p^t Ee (ee)*		Yellow	Yellow	Ivory	Bronze	Crimson, Magenta, Bronze, Pink
Y-46 (yellow) *NN ss II p^r p^r ee*			Yellow	Pink	Bronze	Bronze, Pink
Margaret (ivory) *NN SS ii PP ee*				Ivory	Pink	Pink
Y-19B (bronze) *NN ss II PP ee*					Bronze	Bronze, Pink
Y-19P (pink) *NN Ss II PP ee*						Bronze, Pink

TABLE 2.—SYNONYMY OF TERMS USED TO DESCRIBE THE PRINCIPAL FLOWER COLOUR PHENOTYPES OF *A. majus*

The Authors	Wheldale (14)	Geissman et al. (5)	Haney (7)	Baur (2)
white	white	albino	white	weisz
yellow	yellow	yellow	yellow	gelb
ivory	ivory	ivory	ivory	elfenbein
bronze	bronze	yellow-orange	bronze	eosinrot auf gelb
pink	rose doré	pink	pink	eosinrot auf elfenbein
crimson	crimson	orange-red	red	fuchsinrot auf gelb
magenta	magenta	magenta	purple	fuchsinrot auf elfenbein

TABLE 3.—SYNONYMY OF SYMBOLS USED FOR FLOWER COLOUR GENES OF *A. majus*

Kuckuck and Schick (8)	Wheldale (12)	Baur (1)	Baur (2)	Geissman et al. (5)	Haney (7)
niv (*nivea*)	*y*	*b*	*b*	*n*	*w*
inc (*incolorata*)	*l*(1)	*f*	*f*	*p*	*iv*
sulf (*sulfurea*)	*i*	*c*	*c*	*y*	*y*
eos (*eosina*)	*b*	*a*	*a*	*m*	*dil*
pal (*pallida*)	*d, s*	*m, r*	*pal, x, i*		

(1) *l=r* of Wheldale (14) and Dayton (4)

Nomenclature

Table 2 gives the synonymy, established from published data, of terms used by other workers to describe the principal flower colour phenotypes of *A. majus*. The authors have followed the system of Wheldale, except that "pink" is used rather than "rose doré", the name of a pink horticultural variety.

Unfortunately, *Antirrhinum* geneticists have used a multiplicity of symbols for the same flower colour genes. Table 3 lists the synonyms of the gene symbols *niv*, *sulf*, *inc*, and *eos*, which Böhme and Schütte (3) determined from evidence in the literature, and the synonyms of *pal* which we have added.

The symbols of Kuckuck and Schick (8) are abbreviations of Latin descriptions of the recessive phenotypes (Table 3) and hence have international usefulness. We favour this system because it is complete for all known *Antirrhinum* genes (11) and because it is used in most of the literature on *Antirrhinum* genetics for the past 30 years. For convenience, only the initial letter of each symbol is used in some of the tables.

PART I. FLOWER COLOUR GENES INVESTIGATED

Parent Material

Initially, three commercial varieties and three breeders' selections of *A. majus* were selfed and, at the same time, crossed in 15 combinations (Table 4).

TABLE 5.—THE SEGREGATION OF F_2 POPULATIONS FOR CYANIC VS. ACYANIC FLOWERS (Expected results are in parentheses)

Cross	F_1 colour	F_1 genotype	Culture number	F_2 segregation		P value
				cyanic	acyanic	
Fran. Sch. x Sut. Yel.	yellow	*ii Pp*^{t}	55–66	0 (0)	90 (∞)	—
Fran. Sch. x Margaret	ivory	*ii PP*	55–55	0 (0)	182 (∞)	—
Sutton's Yel. x Y-46	yellow	*Ii p*^{t}*p*^{r}	55–49	0 (0)	84 (∞)	—
Sutton's Yel. x x Margaret	ivory	*ii Pp*^{t}	55–75	0 (0)	176 (∞)	—
Margaret x Y-19B	pink	*Ii PP*	55–36	147 (146.25)	48 (48.75)	.90–.95
Fran. Sch. x Y-19B	crimson	*Ii PP*	55–60	215 (217.5)	75 (72.5)	.90–.95
Fran. Sch. x Y-19B	crimson	*Ii PP*	55–62	231 (237.0)	85 (79.0)	.30–.50
Fran. Sch. x Y-19P	crimson	*Ii PP*	55–57	508 (516.75)	181 (172.25)	.30–.50
Fran. Sch. x Y-19P	magenta	*Ii PP*	55–59	206 (193.5)	52 (64.5)	.05–.10
Y-46 x Y-19B	bronze	*II Pp*^{r}	55–45	244 (223.5)	54 (74.5)	.001–.01
Y-46 x Y-19P	bronze	*II Pp*^{r}	55–42	209 (210.75)	72 (70.25)	.80–.90
Y-46 x Y-19P	pink	*II Pp*^{r}	55–44	171 (174.0)	61 (58.0)	.50–.70
Y-46 x Fran. Sch.	crimson	*Ii Pp*^{r}	55–64	127 (131.1)	106[1] (101.9)	.50–.70
Y-46 x Margaret	pink	*Ii Pp*^{r}	55–40	131 (131.1)	102 (101.9)	.99
Sutton's Yel. x Y-19B	bronze	*Ii Pp*^{t}	55–80	166 (173.25)	142 (134.25)	.30–.50
Sutton's Yel. x Y-19B	bronze	*Ii Pp*^{t}	55–81	181 (175.5)	131 (136.5)	.50–.70
Sutton's Yel. x Y-19P	pink	*Ii Pp*^{t}	55–79	164 (155.8)	113 (121.2)	.30–.50

[1] The yellow flowers of 18 of these were speckled with crimson.

With the exception of Sutton's Intermediate Yellow, one plant of each variety was used for these and all later crosses. A different plant of Sutton's Yellow was used in each of the four crosses (Table 4) and the results show that the plant crossed with Y-19B was *eos eos* whereas the plant crossed with Y-19P was *Eos eos*. The latter was used in all subsequent crosses. The two plants, Y-19B and $Y_1$9P, are sister seedlings selected from the progeny of a pink plant selfed.

Two Genes Controlling Anthocyanin Synthesis

Unexpectedly, the F_1 of ivory Margaret x yellow Y-46 was pink and the F_1 of yellow Francis Schlegel x Y-46 was crimson (Table 4). This is explained by the F_2 results (Table 5) which show that two genes (later identified as *Inc* and *Pal*) are necessary for anthocyanin synthesis. Thus, the acyanic F_1's bred true, whereas cyanic F_1's gave 3:1 ratios in the F_2 when either gene was segregating and 9:7 ratios when both were segregating. These and later results agree with the *Inc* and *Pal* genotypes assigned to the parents (Table 4) and to the F_1's (Table 5).

In early work with *eos eos* material we could make no distinction between the activities of the two anthocyanin genes. It seemed possible, however, that one was *inc* and the other *pal*$^{t\,incta}$ (*pal*$_1$ of Baur, 2). Of the two, Baur noted that *inc* was more commonly responsible for acyanic varieties. Therefore, we crossed ten acyanic horticultural varieties to both Margaret and Y-46 and found, as Table 6 shows, that the gene we call *inc* is indeed more commonly responsible for the acyanic phenotypes of this sample than is *pal*.

We have conclusive evidence that *pal* is our other anthocyanin gene and that two alleles are present. The allele from Sutton's Yellow is *pal*$^{t\,ub}$

TABLE 6.—PHENOTYPES OF F_1 POPULATIONS FROM CROSSES BETWEEN MARGARET AND Y-46, AND TEN ACYANIC HORTICULTURAL VARIETIES

Variety	Margaret (*inc inc Pal Pal*)	Y-46 (*Inc Inc pal pal*)	Variety Genotypes
Ethel (yellow)	ivory	bronze	*ii PP NN ss ee*
Junglewood (yellow)	ivory	bronze	*ii PP NN ss ee*
Klondyke Supreme (yellow)	ivory	crimson	*ii PP NN ss EE*
Golden Glory (yellow)	ivory	crimson	*ii PP NN ss EE*
Yellow Giant (yellow)	ivory	crimson	*ii PP NN ss EE*
Lucky Strike (ivory)	—	pink	*ii PP NN SS ee*
Yellow Jacket (yellow)	ivory	bronze	*ii PP NN ss ee*
Ceylon Court (yellow)	ivory	yellow with pink stripes, crimson, bronze	*ii Pp NN ss Ee*
Schlegel's Early White (ivory)	ivory	ivory	*ii pp NN SS ee*[1]
Golden Monarch (yellow tubocolorata)	magenta	yellow with crimson basal spot and stripes	*II pp NN ss EE*

[1] The F_1 of Schlegel's Early White x Y-19P was pink.

but in our material the characteristic spot of anthocyanin at the base of the corolla (*tubocolorata*) develops only in *Eos* plants. Flowers of *Inc Inc* pal^{tub} pal^{tub} *eos eos* plants are acyanic. Similarly, the expression of the *pal* allele of Y-46 (which we call pal^{rec}) depends on whether the plant is *Eos*—or *eos eos*. Our *Inc*—pal^{rec} pal^{rec} *Eos*—plants are marked with distinct but irregular stripes of magenta (*recurrens*) under all cultural conditions. On the other hand, *Inc*—pal^{rec} pal^{rec} *eos eos* plants grown in the field in summer have acyanic flowers except for a rare pink fleck, whereas flowers that develop in a cool greenhouse frequently show pink stripes as did some of the F_1 plants in Table 5. In fact, the phenotypes of our pal^{rec} in *eos eos* plants resemble those ascribed to pal^{mac} (8; 2, factor i_1). Possibly the distinction between pal^{rec} and pal^{mac} depends on the presence or absence of *Eos*. We use the designation pal^{rec} because Baur (2) stated that pal^{rec} is the commoner of the two alleles and is the one found in striped horticultural varieties.

As an example of the evidence that our pal^{rec} and pal^{tub} are allelic, two crosses raised in the field in 1958 are interesting. The common parent, *Inc Inc* pal^{rec} pal^{tub} *Eos eos*, had flowers with both specks and the basal spot on a yellow background. The cross pal^{rec} pal^{tub} *Eos eos* × pal^{rec} pal^{rec} *eos eos* gave 22 speckled yellow, 23 speckled yellow with the basal spot, and 46 pure yellow plants. The cross pal^{rec} pal^{tub} *Eos eos* × pal^{tub} pal^{tub} *eos eos* gave 24 speckled with the basal spot, 19 yellow with the basal spot, and 59 pure yellow plants (Expected, 25.5: 25.5: 51; $.30 > P > .20$).

It is relatively easy to equate our *inc* with *p* of Geissman and co-workers and *iv* of Haney. Seikel and Geissman (10) named the yellow variety Ball's Gold as the source of their *p*. Haney (7) showed that both Ball's Gold and Margaret were *iv iv*. We found that Margaret is *inc inc*. Therefore our *inc*, Haney's *iv* and Geissman's *p* are identical.

Geissman and co-workers (5), in an attempt to equate their *P* with one of Wheldale's genes, suggest as the most likely interpretation that *P* equals Wheldale's *D*. It is now possible to show that this is not so. In both

Eos—and *eos eos* material, the flowers of Wheldale's *dd* plants were not acyanic, but rather developed tinges of anthocyanin (flushes). Baur (2) concluded, therefore, that Wheldale's *d* was pal^{car} (pal_1). There is additional evidence that Wheldale's *d* is a *pal* allele. Wheldale (12) showed that her factor *S*, which produced stripes of anthocyanin on otherwise acyanic flowers, was allelomorphic to *d*. In *Eos* material the stripes produced by *S* were well developed, whereas, Wheldale (13) reported that, as far as she knew, striped forms did not occur in bronze and pink. Thus *S* appears to equal our pal^{rec}. Therefore Wheldale's *d*, which is allelic to S, cannot be the *p* of Geissman and co-workers.

Anthocyanin Dilution Factor

Part 2 of this paper is concerned with the influence of *inc*, *pal* and a third gene, *diluta*, on the intensity of anthocyanin pigmentation in bronze and pink flowers. Bronze and pink are weakened uniformly throughout the corolla in *dil dil* plants. The varieties Y-19B, Y-19P, Margaret and Sutton's Yellow proved to be *Dil Dil*, whereas Y-46 was *dil dil*.

The material to prove by genetic test that our *dil* equals the *dil* of Kuckuck and Schick (8) and Baur (1;2, factor *L*) was unavailable. The phenotypes are similar except that those authors describe *Dil* as incompletely dominant, whereas our *Dil* was almost completely dominant in the material reported in part 2. At first we thought that we had a new gene and named it *rediluta* (9). However, evidence obtained in 1958 shows that our *Dil* is sometimes incompletely dominant also. Hence we feel that a new name is not justifiable.

PART II. INHERITANCE STUDIES ON THE SHADES OF BRONZE AND PINK

Materials, Methods and Procedures

Progenies used for inheritance studies on the shades of bronze and pink are descended from eight F_1 plants. The phenotypes, genotypes and origins of these F_1's are listed in Table 7. Because all the F_1's are *Niv Niv eos eos*, these genes are henceforth ignored.

The F_1 plants were selfed and seven F_2's were grown in the field in 1955. Unfortunately, because of the late planting date, only a fraction of each

TABLE 7.—PHENOTYPES, GENOTYPES AND ORIGINS OF THE EIGHT F_1 PLANTS FROM WHICH ARE DESCENDED ALL PROGENIES USED FOR INHERITANCE STUDIES ON THE SHADES OF BRONZE AND PINK

Culture number	Phenotype	Genotype	Origin
55-36	pink 3	*Inc inc Pal Pal Dil Dil Sulf sulf*	Y-19B x Margaret
55-40	pink 2	*Inc inc Pal* pal^{rec} *Dil dil Sulf sulf*	Margaret x Y-46
55-42	bronze 3	*Inc Inc Pal* pal^{rec} *Dil dil sulf sulf*	Y-19P x Y-46
55-44	pink 3	*Inc Inc Pal* pal^{rec} *Dil dil Sulf sulf*	Y-19P x Y-46
55-45	bronze 3	*Inc Inc Pal* pal^{rec} *Dil dil sulf sulf*	Y-19B x Y-46
55-79	pink 2	*Inc inc Pal* pal^{tub} *Dil Dil Sulf sulf*	Sutton's Yellow x Y-19P
55-80	bronze 2	*Inc inc Pal* pal^{tub} *Dil Dil sulf sulf*	Sutton's Yellow x Y-19B
55-81	bronze 2	*Inc inc Pal* pal^{tub} *Dil Dil sulf sulf*	Sutton's Yellow x Y-19B

population flowered before frost. However, plants with a range of colour shades were selected from those that did flower. These selections were propagated vegetatively in the greenhouse and were selfed to give F_3 seed.

In 1956, the seven F_2 populations were grown again in the field from residual seed and, at the same time, 16 F_3 families were planted side by side with their respective F_2's. That year the majority of plants flowered before frost.

The F_2 populations segregated into what, to the casual observer, seemed to be a continuum of colour shades from very pale to very dark. However, some F_3 families bred true for one shade or gave simple segregations. From these, we established a shade classification and formulated a genetic interpretation that explained most but not all of the data. Additional progenies (18 in 1957 and 10 in 1958) were grown in the field to test critical points in our interpretation. The 1957 and 1958 results approached expectations more closely than those of 1956. This is probably a reflection of both greater skill at scoring the shades and also the fact that in 1957 and 1958 almost all plants flowered before frost.

As explained in Part I of this report, it is impossible to determine from F_2 and F_3 phenotypes whether a family is segregating for *Inc* and *inc* or *Pal* and *pal*. Accordingly, whenever the *inc-pal* genotype of a parent plant could not be established from its pedigree, the genotype was determined by crossing the plant to both Margaret and Y-46.

The observed and expected results from each segregating population were compared, using the chi-square test. Only the P values were tabulated. When the expected class contained five plants or fewer that class was pooled with the most similar class before the chi-square test was run.

Classification of Shades

Four shades of bronze and four of pink were recognized by eye. The intensity of pigmentation increases at approximately equal intervals from shade 1 to shade 4. Variation of colour intensity exists within each shade and sometimes we encountered plants with flower colours on the borderline between two shades. Shade variation caused by age of the flowers and by differences in yellow pigment were overcome by using large buds that were just about to open. Critical scoring was done in diffuse light. To ensure uniformity from year to year, cuttings of parent plants of each family were planted in the field adjacent to the progeny and served as colour standards. Undoubtedly, scoring errors were made, but the majority of flowers were assigned to one class or another with confidence.

We have attempted to match the *Antirrhinum* flower shades with colours published in the Horticultural Colour Chart of the Royal Horticultural Society. None of the bronze and pink shades is exactly duplicated in the Colour Chart but the closest approximations give an indication of the differences between our shades. The colour approximations for the shades of almost opened buds are:

bronze 1 and pink 1	*ca.* Orient pink 416
bronze 2 and pink 2	*ca.* Delft rose o20/$_3$
bronze 3 and pink 3	*ca.* Delft rose o20/$_1$
bronze 4 and pink 4	*ca.* Delft rose o20

The colour approximations for the outer face of the tips (palate spot ignored) of 1-day old open flowers are:

bronze 1	*ca.*	Aureolin 3
bronze 2	*ca.*	Saffron yellow $7/_1$
bronze 3	*ca.*	Persimmon orange $710/_3$
bronze 4	*ca.*	Burnt orange o14
pink 1	*ca.*	Spinel pink $o625/_3$
pink 2	*ca.*	Spinel pink $o625/_1$
pink 3	*ca.*	Carmine $21/_1$
pink 4	*ca.*	Cherry $722/_3$

Culture Numbers

The results are tabulated according to the complexity of segregation, rather than by pedigree. However, the pedigrees of most parent plants are given by the culture numbers. For example, the F_2 plant 55-40-07 is a selection from the progeny of plant 55-40 selfed; the F_3 plant 55-40-07-01 is a selection from the progeny of 55-40-07 selfed. The numbers of five plants (they begin with 57-) do not follow this system. Their pedigrees are explained in the text.

The Gene Dil

Dil is responsible for the difference between *Inc Inc Pal Pal* plants that breed true for shade 3 (Table 8a) and those that breed true for shade 4 (Table 8b, c, d). Table 8e shows that progenies from *Dil dil* plants segregated in 3:1 ratios. Most of the data obtained in 1956 and 1957 indicate that *Dil* is almost completely dominant. In 1958, however, F_2's from 55-80-02 × 55-42-04-03 (57-77-01 and 57-77-02) gave 1 bronze 3: 2 light bronze 4: 1 dark bronze 4. These bronze 4's were not scored with absolute confidence but their presence shows that *Dil* is not completely dominant under all conditions.

TABLE 8.—*Antirrhinum* PROGENIES, OBTAINED BY SELFING, THAT CONTAINED NO ACYANIC SEGREGATES. THEORETICAL RATIOS ARE GIVEN OPPOSITE THE GENOTYPES; EXPECTED ARE IN PARENTHESES

Genotype and culture number of parent plant	Phenotype of parent	Phenotypes in progeny			P value	Year
		bronze 3	bronze 4	pink 4		
(a) *II PP dd ss*		∞				
55–42–04–03	bronze 3	63				1957
55–42–04–04	bronze 3	65				1957
55–42–04–06	bronze 3	29				1957
(b) *II PP DD ss*			∞			
Y-19B	bronze 4		56			1957
55–42–01	bronze 4		94			1956
55–42–02	bronze 4		344			1956
55–45–02	bronze 4		260			1956
55–80–02	bronze 4		68			1957
(c) *II PP DD SS*				∞		
55–44–01	pink 4			164		1956
(d) *II PP DD Ss*			1	3		
Y-19P	pink 4		12(16.5)	54 (49.5)	.20–.30	1957
55–40–01	pink 4		18(19.5)	60 (58.5)	.50–.70	1956
(e) *II PP Dd ss*		1	3			
55–45–01	bronze 4	25 (23.25)	68 (69.75)		.50–.70	1956
55–45–03	bronze 4	74 (68.5)	200 (205.5)		.30–.50	1956
57–77–01	bronze 4	43 (45)	137 (135)		.70–.80	1958
57–77–02	bronze 4	50 (48.25)	143 (144.75)		.70–.80	1958

TABLE 9.—FLOWER COLOUR SEGREGATIONS OBSERVED IN F_2 AND F_3 POPULATIONS. THEORETICAL RATIOS ARE GIVEN OPPOSITE THE GENOTYPES; EXPECTED RESULTS ARE IN PARENTHESES

Genotype and culture number of parent plant	Phenotype of parent	Phenotypic segregation in selfed progeny							P value	Year
		yellow	bronze 2	bronze 3	bronze 4	ivory	pink 2	pink 3	pink 4		
(a) *Ii PP DD ss*	bronze 3	29(25.5)		48 (51)	25 (25.5)					.70–.80	1957
55–80–04		1		2	1						
Ii PP DD Ss	pink 3	17 (21.9)		46 (43.8)	22 (21.9)	68 (65.6)		133 (131.3)	64 (65.6)	.80–.90	1956
55–40–02	pink 3	15 (12.2)		26 (24.4)	14 (12.2)	33 (36.6)		81 (73.1)	26 (36.6)	.30–.50	1958
55–36											
(b) *Ii PP Dd Ss*	pink 3	15 (11.5)	7 (5.8)	33 (20.1)	9 (8.6)	30 (34.5)	10 (17.3)	56 (60.4)	24 (25.9)	.05–.10	1958
57–A35–01	pink 3	8 (11.1)	8 (5.6)	21 (19.5)	9 (8.3)	34 (33.4)	11 (16.7)	62 (58.4)	25 (25.0)	.70–.80	1958
57–A35–02		4	2	7	3	12	6	21	9		
(c) *Ii Pp'''' DD ss*	bronze 3	22 (18)		33 (36)	17 (18)					.50–.70	1956
55–40–05	bronze 3	64 (65)		134 (130)	62 (65)					.80–.90	1956
55–44–03		1		2	1						
Ii Pp'''' DD ss	bronze 3	18 (25.5)		58 (51)	26 (25.5)					.20–.30	1957
55–80–03											
(d) *Ii Pp'''' dd ss*	bronze 2	83 (77.75)	2	85 (77.75)						.30–.50	1956
55–42–04	bronze 2	70 (73.5)	143 (155.5)	81 (73.5)						.50–.70	1956
55–44–04	bronze 2	20 (24.75)	143 (147)	26 (24.75)						.50–.70	1957
55–45–07	bronze 2	22 (23.25)	53 (49.5)	25 (23.25)						.90–.95	1958
57–A33–01	bronze 2	25 (21.5)	46 (46.5)	23 (21.5)						.50–.70	1958
57–A33–02			38 (43)								
(e) *Ii Pp'''' Dd ss*	bronze 3	72 (70.3)	2	106 (122.9)	74 (52.7)					.001–.01	1956
55–42	bronze 3	54 (74.5)	29 (35.1)	130 (130.4)	56 (55.9)					< .001	1956
55–45		4	58 (37.2)	7	3						
Ii Pp'''' Dd Ss	pink 3	19 (14.5)	2	32 (25.4)	9 (10.9)	42 (43.5)	6	21	9	.001–.01	1956
55–44	pink 3	12 (12.4)	11 (7.3)	17 (21.7)	8 (9.3)	49 (37.1)	36 (21.8)	59 (76.1)	24 (32.6)	.30–.50	1956
55–44–02			7 (6.2)				19 (18.6)	65 (65.0)	21 (27.8)		

TABLE 10.—FLOWER COLOUR SEGREGATIONS OBSERVED IN F_2, F_3 AND F_4 POPULATIONS. THEORETICAL RATIOS ARE GIVEN OPPOSITE THE GENOTYPES; EXPECTED RESULTS ARE IN PARENTHESES

Genotype and culture number of parent plant	Phenotype of parent	Phenotypic segregation in selfed progeny										P value	Year
		yellow	bronze 1	bronze 2	bronze 3	bronze 4	ivory	pink 1	pink 2	pink 3	pink 4		
(a) *Ii Pp^{tub} DD ss*		7		4	4	1							
55–80	bronze 2	142 (134.8)		72 (77)	80 (77)	14 (19.2)						.50–.70	1956
55–81	bronze 2	131 (136.5)		61 (78)	101 (78)	19 (19.5)						.01–.02	1956
55–80–05	bronze 2	182 (176.3)		92 (100.8)	98 (100.8)	31 (25.2)						.50–.70	1957
57–A32–01	bronze 2	168 (165.4)		105 (94.5)	85 (94.5)	20(23.6)						.30–.50	1958
Ii Pp^{tub} DD Ss		7		4	4	1	21		12	12	3		
55–79	pink 2	25 (30.3)		11 (17.3)	20 (17.3)	3 (4.3)	88 (90.9)		52 (51.9)	66 (51.9)	12 (13)	.30–.50	1956
(b) *Ii Pp^{ree} dd ss*		7	4	4	1								
55–40–04–01	bronze 1	58 (70)	47 (40	45(40)	10 (10)							.20–.30	1957
55–40–04–02	bronze 1	60 (70.4)	40 (40.3)	50 (40.3)	11 (10)							.20–.30	1957
55–40–07–01	bronze 1	80 (85.3)	51 (48.8)	47 (48.8)	17 (12.2)							.30–.50	1957
55–40–07–02	bronze 1	80 (83.6)	50 (47.8)	49 (47.8)	12 (11.9)							.95–.99	1957
Ii Pp^{ree} dd SS							7	4	4	1			
55–40–11	pink 1						163 (143.5)	78 (82)	71 (82)	16 (20.5)		.10–.20	1957
Ii Pp^{ree} dd Ss		7	4	4	1		21	12	12	3			
55–40–12	pink 1	36 (33.5)	16 (19.1)	16 (19.1)	4 (4.8)		98 (100.4)	54 (57.4)	58 (57.4)	24 (14.3)		.20–.30	1957
(c) *Ii Pp^{ree} Dd ss*		28	4	16	13	3							
55–40–07	bronze 2	116 (120.3)	14 (17.2)	81 (68.8)	61 (55.9)	3 (12.9)						.02–.05	1956
55–40–08	bronze 2	114 (144.4)	17 (20.6)	105 (82.5)	49 (67)	15 (15.5)						.02–.05	1956
Ii Pp^{ree} Dd Ss		28	4	16	13	3	84	12	48	39	9		
55–40	pink 2	18 (25.5)	4 (3.6)	15 (14.6)	14 (11.8)	1 (2.7)	84 (76.5)	10 (10.9)	35 (43.7)	39 (35.5)	13 (8.2)	.30–.50	1956
55–40–04	pink 2	45 (38)	3 (5.4)	24 (21.7)	17 (17.6)	3 (4.1)	117 (113.9)	20 (16.3)	62 (65.1)	45 (52.9)	11 (12.2)	.80–.90	1956

Inc inc Segregations

Crossing acyanic segregates to the testers Margaret and Y-46 showed that *Inc* rather than *Pal* was responsible for the 1:2:1 ratios listed in Table 9a. Thus *Inc inc Pal Pal Dil Dil* plants, like *Inc Inc Pal Pal dil dil* plants have shade 3 flowers. To be sure, such *Inc inc* plants are often slightly paler than the *dil dil* plants but the difference cannot be scored accurately and both fall within the range of shade 3.

Influence of Sulf

The segregations from two *Sulf sulf* parents listed in Table 9a show that pink 3 and pink 4 are the equivalents of bronze 3 and bronze 4 with respect to *Inc-Pal-Dil* genotypes. Similarly, two *Sulf sulf* pink 4 plants (Table 8d) gave pink 4 and bronze 4 progeny. Thus *Sulf* segregation does not influence the shades of pink and bronze.

Pal pal Segregations

Table 9 gives the results from selfing eight *Pal pal* plants. When *dil dil* (Table 9d) is substituted for *Dil Dil* (Table 9c), the cyanic classes are shades 2 and 3 instead of shades 3 and 4. Flowers of *Inc Inc Pal* pal^{tub} (and pal^{rec}) *Dil Dil* plants are indistinguishable from those of *Inc Inc Pal Pal dil dil* plants, and are the same or slighly darker than those of *Inc inc Pal Pal Dil Dil* plants.

Pal pal Dil dil Segregations

Three of the four *Pal pal Dil dil* segregations (Table 9e) deviate significantly from the expected results. We do not have a satisfactory genetic explanation for this. Possibly linkage is involved. We have evidence from a test cross of 36 per cent crossing-over between *Pal* and *Dil*. Coupling of this intensity gives expected ratios that satisfactorily fit the results from 55-42 ($.30 > P > .20$) and from 55-44-02 ($.20 > P > .10$) but for 55-44 and 55-45 the deviations are still highly significant. However, the three deviating F_2's agree somewhat with the expected results in that the required shades are present in roughly the expected proportions. In addition,

TABLE 11.—RESULTS FROM CROSSES BETWEEN SHADE 1 PLANTS AND SHADE 4 PLANTS. THEORETICAL RATIOS ARE GIVEN OPPOSITE THE GENOTYPES; EXPECTED RESULTS ARE IN PARENTHESES.

Cross	Progeny			P value
	pink 2	pink 3	pink 4	
Ii Pp^{rec}*dd S-* x *II PP DD S-* (pink 1) (pink 4)	1	2	1	
55-40-11 x 55-40-01-02	46 (48.75)	100 (97.5)	49 (48.75)	.80–.90
55-40-12-x-55-40-01-01	51 (50.5)	88 (101.0)	63 (50.5)	.05–.10
	bronze 2	bronze 3	bronze 4	
Ii Pp^{rec} *dd ss* x *II PP DD ss* (bronze 1) (bronze 4)	1	2	1	
55-40-04-01 x 55-40-01-03	26 (25.5)	47 (51.0)	29 (25.5)	.50–.70
55-40-07-01 x 55-40-01-03	20 (24)	46 (48)	30 (24)	.30–.50

progenies derived from the deviating families behaved as expected. These are three F_3's (Tables 8b; 9d) and three F_4's (Table 8a) from 55-42, and four F_3's from each of 55-44 (Tables 8c; 9c, d, e) and 55-45 (Tables 8b, e; 9d).

Inc inc Pal pal Segregations

Table 10 gives the results from selfing eleven *Inc inc Pal pal* plants. All but one of the progenies agree with the expected results. The cyanic classes are shades 2, 3 and 4 from *Dil Dil* plants and shades 1, 2 and 3 from *dil dil* plants. These segregations show that *Inc inc Pal pal Dil Dil* plants have the shade 2 flowers (Table 10a) and *Inc inc Pal pal dil dil* plants have shade 1 flowers (Table 10b).

Table 11 gives the results from crossing *Inc inc Pal pal dil dil* (shade 1) plants with *Inc Inc Pal Pal Dil Dil* (shade 4) plants.

Genotypes of the shade 1 parents were established from the results given in Table 10b; the shade 4 parents were selected from the progeny of 55-40-01 (Table 8d). The ratio of 1 shade 2: 2 shade 3: 1 shade 4 shows that the intensifying effects of *Inc* and *Pal* are similar.

Inc inc Pal pal Dil dil Segregations

Four cyanic shades appear in progenies segregating for all three anthocyanin genes (Table 10c). Two of the segregations deviate significantly from the expected results and linkage between *Pal* and *Dil* will not account for the deviations of 55-40-08. However, repulsion with 36 per cent crossing-over does fit the data from 55-40-07 ($.20 > P > .10$) and coupling gives excellent fits for 55-40 ($.80 > P > .70$) and for 55-40-04 ($.70 > P > .50$).

Dil Constitution of Original Parents

Both Y-19B and Y-19P bred true for shade 4 (Table 8b, d) and hence are *Dil Dil*. Both were crossed to *Inc Inc Pal Pal dil dil* plants and gave shade 4 F_1's as expected. Similarly, Sutton's Yellow is *Dil Dil*. When crossed with Y-19P (55-79, 57-A32-01) and with Y-19B (55-80, 55-81) the F_1's bred true for *Dil* (Table 10a).

The original plant of Margaret is also *Dil Dil*. It was crossed with 55-42-04-04 (*Inc Inc Pal Pal dil dil*) giving 69 pink 3 plants. Two of these, 57-A35-01 and 57-A35-02, were selfed and the F_2's raised in 1958 (Table 9b) along with the F_2 of Y-19B $\times$ Margaret (55-36, Table 9a). The three families gave the results expected.

In contrast, Y-46 proved to be *dil dil*. It was crossed to 55-42-04-04 (*Inc Inc Pal Pal dil dil*) giving 23 bronze 2 plants. Two of these, 57-A33-01 and 57-A33-02, gave the simple F_2 segregations that were expected (Table 9d).

CONCLUSIONS

The foregoing data show that the formation of anthocyanin in flowers of *eos eos* genotypes depends on the presence of one allele each of both *Inc*

and *Pal*. Additional dominant alleles of *Inc*, *Pal* and of *Dil* increase the intensity of the pigmentation. The data indicate that the four shades of pink and bronze flowers have the following genotypes:

Shade 1=*Inc inc Pal pal dil dil*
Shade 2=*Inc inc Pal pal Dil Dil* (and *Dil dil*)
=*Inc inc Pal Pal dil dil*
=*Inc Inc Pal pal dil dil*
Shade 3=*Inc inc Pal Pal Dil Dil* (and *Dil dil*)
=*Inc Inc Pal pal Dil Dil* (and *Dil dil*)
=*Inc Inc Pal Pal dil dil*
Shade 4=*Inc Inc Pal Pal Dil Dil* (and *Dil dil*)

These genotypes were studied in both *Sulf*—and *sulf sulf* plants and with both pal^{rec} and pal^{tub}. However, the effect of pal^{tub} is known only in *Dil Dil* material.

The dosage effects of the three genes are additive. The effect of one *Inc* allele approximates that of one *Pal* allele or that of one or two *Dil* alleles. If we give the numerical value 1 to each dominant allele of *Inc* and *Pal* and to both *Dil dil* and *Dil Dil*, shade 1 plants have a dose of two, shade 2 plants a dose of three, shade 3 plants a dose of four and shade 4 plants a dose of five.

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CONTROL OF THE EASTERN FIELD WIREWORM, *LIMONIUS AGONUS* (SAY), IN EARLY POTATOES IN ONTARIO BY APPLICATION OF INSECTICIDES TO THE SOIL[1]

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ABSTRACT

When applied as water suspensions at 5.0 lb. per acre for control of a very heavy population of the eastern field wireworm, dieldrin[2], heptachlor, and aldrin prevented re-infestation for at least 3 years after application, and chlordane for 2 years. Only dieldrin and heptachlor gave 95 per cent, or more, marketable potatoes in the year of application. BHC containing 12, 90, and 99 per cent gamma isomer at 0.75 lb. of the gamma isomer, parathion at 5.0 lb., and DDT at 10.0 lb. per acre gave inadequate protection, or were effective for only 1 to 2 years. Against a moderate infestation of wireworms, all treatments except BHC containing 90 per cent gamma isomer and DDT protected the potatoes satisfactorily in the year of application. BHC tainted tubers up to 2 years after the insecticide was applied. Adequate protection was generally provided when the wireworm infestation averaged no more than 1 larva per bait. The insecticides were applied once in plots in one of three adjoining ranges in 1951, 1952, and 1953, respectively. Potatoes were grown in the treated ranges until 1954. The treatments were evaluated on the basis of tuber quality, and numbers of wireworms taken in whole-wheat flour baits.

INTRODUCTION

The eastern field wireworm, *Limonius agonus* (Say), is a serious pest of potatoes in the sandy soils of southwestern Ontario. The intensive cultivation practised in this area favours this species, as the females prefer to oviposit in loose, cultivated soil (10). Infestations became so severe that production of high-grade tubers was impossible in some districts.

Control of this wireworm was unsatisfactory before organic insecticides were introduced. From 1945 to 1950, experimental work in Canada, the United States and elsewhere showed that various species of wireworms, including *L. agonus*, could be controlled by treating the soil with one of many organic insecticides (1, 2, 15, 16, 17, 19). A few insecticides, however, tainted root crops. It was feared that the insecticide residues in the crops might also constitute a health hazard.

In each of the years 1951 to 1953, single applications of nine insecticides were applied to the soil in one of three contiguous ranges for control of the wireworm attacking early potatoes. Potatoes were grown in the plots until 1954. This is a report on the effects of these treatments on wireworm injury to potatoes, wireworm population, and growth, yield, and flavour of the potatoes. The insecticide residues in or on the tubers will be reported elsewhere.[3]

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[1] Contribution No. 3903, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Ont.; from a thesis submitted in partial fulfilment of requirements for M.Sc. degree at the University of Western Ontario, London, Ont.

[2] Chemical names for insecticides used are given beneath Table 1.

[3] Begg, J. A., P. J. G. Plummer, and H. Konst. Insecticide residues in potatoes after soil treatments for control of wireworms. *Unpublished data.*

MATERIALS AND METHODS

A randomized block experiment was conducted in three adjacent ranges in a field of uniform Berrien sandy loam severely infested with *L. agonus*. The population in the field was the highest encountered by the Chatham laboratory staff in southwestern Ontario. More than 19,200 larvae were taken in 600 baits in the experimental area before application of the treatments.

The ranges each contained four blocks of plots, 20 feet long and 20 feet wide. One range was treated with wettable powder formulations of the insecticides (Table 1) on June 2 and 4, 1951, the second on May 16, 1952, and the third on May 26 and 27, 1953. The treatments, applied as suspensions in 108 gallons of water per acre to newly-ploughed land, were doubled-disked into the soil within 7 hours after application.

Each year until 1955, before and after application of the treatments, counts of wireworms were made in May using a baiting technique. Balls of whole-wheat flour dough, 1.5 inches in diameter, were placed 4 inches deep in five pre-determined sites in each plot. One week later the larvae in each bait were counted and returned to the soil.

Irish Cobbler potatoes were planted in the experimental plots on June 8 in 1951, on May 21 in 1952, on May 27 and 28 in 1953, and on May 12 and 13 in 1954. Routine culture was followed during the growing season.

The effect of the treatments on growth was assessed in July of each year by measuring the height of all potato plants in 14 linear feet in each of the four centre rows in each plot. All tubers from these rows were harvested by hand approximately 12 weeks after planting and the yields recorded.

Wireworm injury was assessed by counting all points where the feeding injury penetrated the skin of the potatoes. This criterion was very stringent as many of the holes would not be evident on peeled potatoes. Protection was considered satisfactory when 95 per cent of the potatoes had one or no punctures. These potatoes were assumed to be marketable. Canada No. 1 grade potatoes must be free from damage which causes a waste of more than 5 per cent of the total weight of the potato, including the peel covering the defective area (22). Rawlins and Davis (16) counted only punctures 0.25 inches or more in depth and rated tubers with two or more holes as unfit for U.S. No. 1 grade.

Significance of the data on damage, growth, yield, and counts of wireworms was determined by analysis of variance. The percentage damage was first transformed to the arcsin scale, and the counts of wireworms to the transformation, $y = \sqrt{\frac{1}{2} + x}$ where x is the number of larvae.

In 1951, 1952, and 1953, taste tests were made on a sample of potatoes taken from four replicates for each treatment. In 1954, these tests were confined to potatoes grown in plots treated with BHC. After the tubers were peeled, sliced, and cooked for 10 minutes in a pressure-cooker, coded samples were rated against a known check by ten members of the laboratory staff. Samples rated as off-flavour by at least two persons were considered to be tainted by a treatment. The method used was similar to that described by Rodriguez and Gould (18). Statistical analysis of the results was not attempted because the laboratory staff had not been trained in taste-testing potatoes (4, 9).

TABLE 1.—PERCENTAGES OF MARKETABLE POTATOES[1] HARVESTED IN VARIOUS YEARS IN PLOTS RECEIVING SINGLE SOIL APPLICATIONS OF WETTABLE POWDERS OF VARIOUS INSECTICIDES, FOR CONTROL OF THE EASTERN FIELD WIREWORM, CHATHAM, ONTARIO

Formulation and toxicant per acre, lb.	1951		1952		1953		1954	
	Percentage	Arcsin transformation	Percentage	Arcsin transformation	Percentage	Arcsin transformation	Percentage	Arcsin transformation
	Treated in 1951							
Dieldrin[4], 25%, 5	95.5[2]	78.3	99.7	88.4	100.0	90.0	100.0	90.0
Heptachlor[5], 25%, 5	98.8	86.9	100.0	90.0	99.3	87.7	97.6	84.2
Aldrin[6], 20%, 5	93.3	77.6	95.3	81.3	99.5	87.9	95.8	78.6
Chlordane[7], 40%, 5	80.0	63.6	98.5	84.0	97.9	84.1	80.4	65.2
BHC, 12% gamma[8], 50%, 0.75 gamma	70.0	57.3	94.4	81.2	72.5	59.9	60.1	51.4
Lindane[9], 25%, 0.75	88.7	70.6	97.4	82.5	67.5	56.3	61.0	51.2
BHC, 90% gamma[8], 50%, 0.75 gamma	81.4	66.1	69.4	56.9	47.5	43.6	57.5	49.4
DDT[10], 50%, 10	64.9	53.8	96.8	80.3	87.4	72.1	68.5	56.0
Parathion[11], 15%, 5	90.0	72.6	41.2	39.8	47.5	43.6	47.9	43.6
Untreated	25.0	29.7	37.5	37.0	31.4	29.9	35.6	36.6
Difference necessary for significance at the 5% level		7.9		14.0		19.1		13.0
	Treated in 1952[3]							
Dieldrin			100.0	90.0	100.0	90.0	100.0	90.0
Heptachlor			100.0	90.0	100.0	90.0	100.0	90.0
Aldrin			100.0	90.0	100.0	90.0	99.8	88.7
Chlordane			97.8	84.0	100.0	90.0	99.3	87.7
BHC, 12% gamma			96.0	79.8	99.5	88.0	97.0	83.0
Lindane			97.3	82.2	99.2	87.5	88.8	70.9
BHC, 90% gamma			89.0	71.0	95.7	78.2	77.8	62.2
DDT			89.6	71.8	90.5	72.9	68.0	56.1
Parathion			98.3	84.7	87.8	69.4	55.1	47.9
Untreated			51.8	45.8	38.7	37.9	66.0	54.8
Difference necessary for significance at the 5% level				7.1		6.3		5.0

[1] Tubers having 0 to 1 wireworm feeding points.
[2] Treatments giving 95% or more marketable potatoes were considered satisfactory.
[3] Formulations and rates as in 1951.
[4] 1,2,3,4,10,10-hexachloro-*exo*-6, 7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene; Julius Hyman Co., Denver, Colo.
[5] 1(or 3a),4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene; Velsicol Corp., Chicago, Ill.
[6] 1,2,3,4,10,10-hexachloro-1,4,4a, 5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene; Julius Hyman Co., Denver, Colo.
[7] 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan; Dow Chemical Co., Midland, Mich.
[8] Mixed isomers of 1,2,3,4,5,6-hexachloro*cyclo*hexane; Canadian Industries Ltd., Montreal, Que.
[9] Gamma isomer of 1,2,3,4,5,6-hexachloro*cyclo*hexane (minimum purity, 99%). Dow Chemical Co., Midland, Mich.
[10] A complex mixture, mainly of 1,1,1-trichloro-2-2-bis(*p*-chlorophenyl) ethane; Canadian Industries Ltd., Montreal, Que.
[11] *O,O*-diethyl *O*-*p*-nitrophenyl phosphorothionate; Cyanamid of Canada, Ltd., Toronto, Ont.

TABLE 1.—PERCENTAGES OF MARKETABLE POTATOES[1] HARVESTED IN VARIOUS YEARS IN PLOTS RECEIVING SINGLE SOIL APPLICATIONS OF WETTABLE POWDERS OF VARIOUS INSECTICIDES, FOR CONTROL OF THE EASTERN FIELD WIREWORM, CHATHAM, ONTARIO
—*Concluded*

Formulation and toxicant per acre, lb.	1951		1952		1953		1954	
	Percentage	Arcsin transformation	Percentage	Arcsin transformation	Percentage	Arcsin transformation	Percentage	Arcsin transformation
	Treated in 1953[2]							
Dieldrin					**100.0**	90.0	**100.0**	90.0
Heptachlor					**97.9**	82.1	**99.7**	88.4
Aldrin					91.3	73.0	94.9	80.7
Chlordane					88.4	73.3	82.8	65.8
BHC, 12% gamma					75.6	60.5	88.7	70.7
Lindane					86.4	69.2	81.4	64.4
Lindane[13], 50%, 0.75					89.7	72.2	76.4	61.3
DDT					55.9	48.7	65.8	54.1
Parathion					83.9	67.5	46.6	42.5
Untreated					24.2	28.6	37.7	37.6
Difference necessary for significance at the 5% level						11.3		8.5

[13] Gamma isomer of 1,2,3,4,5,6-hexachloro*cyclo*hexane (minimum purity, 99%) prepared from lindane of European origin; Chipman Chemicals Ltd., Montreal, Que.

RESULTS AND DISCUSSION

Only dieldrin and heptachlor gave at least 95 per cent marketable potatoes each year potatoes were grown. Aldrin and chlordane gave this percentage protection in 6 and 5 range-years, respectively*. The other materials tested were as effective in only 3 or fewer range-years. Dieldrin gave 100 per cent marketable potatoes in 7 range-years, heptachlor in 4, aldrin in 2, and chlordane in 1. Only dieldrin and heptachlor were satisfactory in the year of application against the very high density of wireworms in the ranges treated in 1951 and 1953. Against the moderate population in the 1952 range, all treatments, with the exception of BHC containing 90 per cent gamma isomer and DDT, provided adequate protection in the year of application (Tables 1 and 2).

These results agree, in general, with those obtained from applications to soil by other workers. Rawlins *et al.* (17), working with *L. agonus* in a fine sandy loam soil, found heptachlor and aldrin superior to chlordane when applied at the same rates for protection of potatoes grown the same year. Kulash and Monroe (11) concluded that heptachlor gave better control of *Melanotus communis* (Gyll.), than did lindane, dieldrin, aldrin, or chlordane, in that order. Stone and Foley (20) found that BHC, DDT, chlordane, aldrin and dieldrin each prevented re-infestation by *Limonius*

* Potatoes were grown 4 years in one range, 3 in the second, and 2 in the third, for a total of 9 range-years.

TABLE 2.—MEAN NUMBERS OF WIREWORMS COLLECTED PER BAIT IN VARIOUS YEARS IN PLOTS RECEIVING SINGLE SOIL APPLICATIONS OF VARIOUS INSECTICIDES, CHATHAM, ONTARIO

Insecticide[2]	1951	1952		1953		1954		1955	
	Mean	Mean	Transformation[1]	Mean	Transformation	Mean	Transformation	Mean	Transformation
	Treated in 1951								
Dieldrin	57.1[3]	2.1	1.51	0.1	0.77	0.0	0.71	0.0	0.71
Heptachlor	39.8	1.0	1.20	0.1	0.74	0.1	0.80	0.5	0.98
Aldrin	49.6	4.0	1.99	0.1	0.77	0.8	1.02	1.0	1.14
Chlordane	36.2	11.9	3.51	0.5	0.99	2.2	1.49	3.7	1.78
BHC, 12% gamma	50.5	10.3	3.06	1.8	1.37	3.3	1.50	5.6	2.44
Lindane	42.7	9.9	3.18	3.3	1.93	10.6	3.29	8.9	3.00
BHC, 90% gamma	42.4	12.9	3.60	1.1	1.22	4.9	2.23	5.2	2.34
DDT	62.3	10.4	3.22	1.3	1.33	3.7	1.98	2.8	1.80
Parathion	46.9	10.0	3.18	4.0	2.03	11.4	3.36	12.8	3.47
Untreated	63.8	27.4	5.25	5.4	2.34	16.7	3.98	10.0	3.18
Difference necessary for significance at the 5% level			1.08		0.68		1.25		1.10
	Treated in 1952								
Dieldrin		18.7[3]		0.0	0.71	0.0	0.71	0.2	0.81
Heptachlor		11.2		0.0	0.71	0.0	0.71	0.1	0.77
Aldrin		18.3		0.0	0.71	0.0	0.71	0.3	0.87
Chlordane		16.3		0.1	0.80	0.0	0.71	0.8	1.08
BHC, 12% gamma		14.3		0.1	0.77	0.2	0.83	1.7	1.43
Lindane		11.7		0.2	0.83	1.6	1.44	2.2	1.62
BHC, 90% gamma		18.6		1.0	1.19	1.5	1.38	0.9	1.18
DDT		14.1		0.9	1.10	1.6	1.33	2.1	1.60
Parathion		11.5		0.4	0.94	2.2	1.59	7.9	2.78
Untreated		14.8		3.0	2.21	6.7	2.59	6.5	2.43
Difference necessary for significance at 5% level					0.70		0.52		0.83

[1] By the formula $\sqrt{1 + x}$
[2] Formulation and rates as in Table 1
[3] Before application of insecticides

TABLE 2.—MEAN NUMBERS OF WIREWORMS COLLECTED PER BAIT IN VARIOUS YEARS IN PLOTS RECEIVING SINGLE SOIL APPLICATIONS OF VARIOUS INSECTICIDES, CHATHAM, ONTARIO—*Concluded*

Insecticide[2]	1951	1952		1953		1954		1955	
	Mean	Mean	Transformation[1]	Mean	Transformation	Mean	Transformation	Mean	Transformation
	Treated in 1953								
Dieldrin				32.8[3]		0.1	0.77	0.0	0.71
Heptachlor				29.1		0.8	1.11	0.0	0.71
Aldrin				26.7		1.1	1.26	1.0	1.20
Chlordane				23.2		1.7	1.60	1.2	1.28
BHC, 12% gamma				32.1		1.2	1.26	1.0	1.18
Lindane				33.2		3.1	1.70	2.1	1.62
Lindane				31.9		2.8	1.81	1.9	1.48
DDT				27.8		3.8	2.04	1.5	1.38
Parathion				25.2		2.4	1.62	4.7	2.25
Untreated				33.2		12.3	3.49	10.0	3.25
Difference necessary for significance at 5% level							0.68		1.40

californicus Mann., for 3 to 4 years. DDT, in general, has not provided adequate control of soil insects (12). Parathion, although effective against wireworms (15), has not been used as a soil insecticide because of its short residual action and high mammalian toxicity (23).

The stabilities of organic insecticides in soil have been investigated by various workers. Foster (5) showed by chemical analysis that DDT remained unchanged over a 4-year period, and after 3 years only about 50 per cent of BHC had disappeared. In a later report, Foster *et al.* (6) concluded that dieldrin is more persistent in soil than aldrin or heptachlor, and that the persistence of an insecticide appeared to be affected by soil type. Residues of BHC, DDT, aldrin, chlordane, and dieldrin were obtained 3 years after application in a sandy loam soil (21). The conversion of aldrin to dieldrin and heptachlor to heptachlor epoxide partially explains the residual properties of these materials (7). The conversion is advantageous since the compounds produced are more toxic and more stable than the parent materials.

When 95 per cent of the potatoes in the treated plots were marketable, the numbers of wireworms taken per bait generally averaged no more than 1. This degree of marketability was obtained in 75.8 and 74.4 per cent of the plots having this population when the censuses were taken the spring before and after the potato crop, respectively. In some cases, small numbers of wireworms caused considerable injury; in others, many larvae

damaged few potatoes. Hawkins (8) also found that the injury caused by a given population of wireworms varied considerably, and concluded that it was impossible to predict crop damage on the basis of larval density alone.

Baiting is a simple method of estimating differences in wireworm densities, but counts made in different years, or at different times in the same year, cannot be directly compared. Numbers coming to a bait depend, in part, on larval activity which, in turn, depends on soil and weather conditions, natural food supply, and size of wireworms. Time of year also is important as the eastern field wireworm is attracted to baits only in the spring and early summer.

In 1951, average height of the potato plants in the plots treated with BHC containing 12 per cent gamma isomer was significantly different, being only 14.5 inches compared to 16.5 inches in the untreated plots. Presumably, this cannot be explained on the basis of treatment as BHC appears to be phytotoxic to potatoes only at very high rates of application (13). Total yields were significantly increased in 1953 from 27.9 pounds in the check plots to 37.1 and 36.9 pounds in the plots treated that year with aldrin and heptachlor, respectively, and in 1954 from 27.7 pounds to 40.5 pounds in the plots treated with heptachlor in 1951. This suggests that yields in infested plots may have been reduced by wireworm feeding.

Only BHC preparations tainted potatoes grown in the experimental plots. BHC containing 12 per cent gamma isomer tainted the tubers grown 2 years after the application. Arnason *et al.* (2) also found off-flavour for 2 years after soil application of BHC. Off-flavour produced by high-gamma BHC was not as pronounced as with BHC containing 12 per cent gamma isomer.

Re-infestation of treated plots probably resulted from oviposition and not from inter-plot movement of the larvae. Large numbers of gravid females could be found in all plots during the flight period. Numbers, however, were progressively smaller from 1951 to 1955. Evidently, the infestations in the environs of the experimental area had been reduced by continued use of seed treatments on field corn, and general soil treatments on potato acreage. Re-infestation of large treated fields would be much slower than in small experimental plots. *L. agonus* females fly only short distances until most of their complement of eggs is deposited in the field in which they developed (3).

The increased protection from the treatments the year after application suggests that control measures should be applied in crop rotations the year preceding a potato crop. Apparently, some time is required for soil insects to contact a lethal concentration when insecticides are imperfectly mixed in the soil by standard cultural practices (14).

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THE USE OF CHEMICALS TO SUPPRESS SUCKER GROWTH ON CIGAR TOBACCO[1]

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ABSTRACTS

Studies on the chemical control of sucker growth on cigar tobacco showed that a mineral oil-water emulsion applied to the stub of the broken stem after the plant was decapitated and diethanolamine salt of maleic hydrazide sprayed on the leaves suppressed sucker growth significantly and increased the yield of the cured crop. Each chemical treatment resulted in a decrease in the percentage content of total alkaloids, nicotine, calcium, and total ash in the cured leaf but had no apparent influence on the content of total nitrogen, nornicotine, potassium, phosphorus, magnesium and chlorine.

INTRODUCTION

In the culture of tobacco, a standard practice, called "topping", is the breaking off of the main stem of the plant at a point which will remove several top leaves and the terminal bud. Following topping, there is a rapid growth of axillary stems, commonly called "suckers", which are then broken off manually. The development of a satisfactory method for the chemical control of sucker growth, thus eliminating the necessity for the costly and laborious manual removal of suckers, would be of great value. However, the practicability of such a method of sucker control is conditioned by any effects it might have on the yield and quality of the crop. Steinberg (5) reported that axillary growth on tobacco was suppressed by certain chemicals but was stimulated by certain others. McEvoy (3, 4) found that several growth-regulating substances suppressed sucker growth effectively but they tended to impair both the yield and quality of the crop.

This paper is a report of an investigation on the use of two chemicals to suppress sucker growth on tobacco and their effects on yield, grade, value, and chemical composition of the cured leaf.

MATERIALS AND METHODS

Resistant Havana 211, a variety of cigar-leaf tobacco, was used in the test. The crop was supplied with 1,000 pounds per acre of a 5-8-10 analysis of tobacco fertilizer. It was transplanted, cultivated, topped, harvested, cured, and graded by the usual methods for cigar-leaf tobacco in Canada. The tobacco was not aged or force sweated (fermented).

The tests were conducted in field plots. Three chemical treatments and a control were set out in quadruplicate on 25-plant plots in randomized blocks. Two chemical substances were tested. Mineral oil (Bayol N 150), mixed with water to form a 50/50 oil-water emulsion, was applied to the stub of the broken stalk immediately after topping at the rate of 3 c.c. per plant. Maleic hydrazide, diethanolamine salt formulation (MH-30)

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TABLE 1.—AVERAGE WEIGHT OF SUCKERS PER PLANT 2 WEEKS AFTER TOPPING, AND CROP DATA OF CIGAR TOBACCO TREATED WITH CHEMICALS TO SUPPRESS SUCKER GROWTH

(2-year average)

Chemical used	Amount applied	Weight suckers per plant (oz.)	Yield per acre (lb.)	Grade index (cents per lb.)	Crop index ($ per acre)
Control		9.8	1419	15.7	223
MH-30	2.25 lb./acre	3.5	1656	15.5	257
MH-30	3.4 lb./acre	3.1	1689	16.4	277
Mineral oil	3 c.c./plant	3.2	1719	15.7	270
L.S.D. (P 5%)		1.9	148	N.S.	N.S.

with a wetting agent in aqueous solution was sprayed on the plants immediately after topping at two rates—2.25 and 3.4 pounds of the actual chemical per acre.

Before harvest, the suckers were removed from both treated and control plants and their fresh weights recorded. After the cured crop was graded, the grade index, yield, and crop index were determined. The grade index and the crop index represent the average value of the crop in cents per pound and the acre value of the crop, respectively.

For chemical analysis, the leaves were divided into three grades: *tops*, *middles*, and *bottoms*. From each grade, a composite sample was made up of 15 leaves from each replicate, selected at random. The midribs were removed from the leaves and discarded. The remaining leaf tissue was dried in a forced-draft oven at 70°C., then ground and stored in air-tight containers. The percentages of the constituents were calculated on a moisture-free basis. Total alkaloids, nicotine, and nornicotine were obtained by the method of Cundiff and Markunas (2), using the Fisher titrimeter manually operated to obtain the equivalence point. The Kjeldahl method for nitrate-containing materials, as described by the A.O.A.C. (1), was used for the determination of total nitrogen. Total ash values were determined on 2-gm. samples by heating at 600°C. for 2 hours. Calcium and potassium were determined volumetrically, using oxalate and cobaltinitrite, respectively, as precipitants and potassium permanganate solution for the final titrations. Phosphorus was determined colorimetrically by the molybdenum blue method with aminonaphthol-sulphonic acid reduction. Magnesium was precipitated as magnesium ammonium phosphate and calculated from a colorimetric determination of phosphorus on the dissolved complex. Chloride was determined by a volumetric method, involving a preliminary precipitation as silver chloride with a measured amount of silver nitrate and back titration with ammonium thiocyanate. Sulphur was determined gravimetrically as barium sulphate.

TABLE 2.—EFFECT OF CHEMICAL TREATMENTS TO SUPPRESS SUCKER GROWTH ON CHEMICAL COMPOSITION OF CIGAR TOBACCO

(Expressed as per cent of dry matter)

Constituent	Leaf grade	Control	MH-30 2.25 lb./ac.	MH-30 3.4 lb./ac.	Mineral oil 3 c.c./plant
% Total alkaloids	Tops	3.69	3.37	3.19	3.67
	Middles	3.82	3.38	3.44	3.65
	Bottoms	3.26	2.44	3.29	2.87
	Weighted Av.	3.65	3.16	3.32	3.46
% Nicotine	Tops	3.56	3.24	3.00	3.55
	Middles	3.62	3.22	3.31	3.45
	Bottoms	3.12	2.23	3.07	2.71
	Weighted Av.	3.48	3.00	3.15	3.29
% Nor-nicotine	Tops	0.12	0.12	0.17	0.11
	Middles	0.19	0.15	0.11	0.17
	Bottoms	0.13	0.18	0.20	0.14
	Weighted Av.	0.15	0.14	0.15	0.14
% Total N.	Tops	4.78	4.90	4.19	4.96
	Middles	4.34	4.21	4.30	4.44
	Bottoms	3.70	3.44	4.59	3.54
	Weighted Av.	4.32	4.24	4.33	4.37
% Ca	Tops	4.08	3.80	4.43	4.00
	Middles	4.96	4.85	4.46	4.80
	Bottoms	5.41	5.35	3.91	5.25
	Weighted Av.	4.80	4.64	4.31	4.68
% K	Tops	3.85	3.79	3.59	3.74
	Middles	3.94	4.07	4.16	4.16
	Bottoms	4.18	4.15	3.88	3.84
	Weighted Av.	3.97	4.00	3.90	3.95
% P	Tops	0.23	0.21	0.17	0.19
	Middles	0.16	0.15	0.16	0.19
	Bottoms	0.15	0.14	0.18	0.14
	Weighted Av.	0.18	0.17	0.17	0.18
% Mg	Tops	0.58	0.60	0.62	0.62
	Middles	0.68	0.68	0.64	0.69
	Bottoms	0.69	0.77	0.61	0.73
	Weighted Av.	0.65	0.68	0.63	0.68

TABEL 2.—EFFECT OF CHEMICAL TREATMENTS TO SUPPRESS SUCKER GROWTH ON CHEMICAL COMPOSITION OF CIGAR TOBACCO—*Concluded*

(Expressed as per cent of dry matter)

Constituent	Leaf grade	Control	MH-30 2.25 lb./ac.	MH-30 3.4 lb./ac.	Mineral oil 3 c.c./plant
% Cl	Tops	0.28	0.28	0.22	0.31
	Middles	0.29	0.25	0.30	0.29
	Bottoms	0.37	0.29	0.40	0.49
	Weighted Av.	0.31	0.27	0.29	0.35
% S	Tops	0.56	0.45	0.42	0.55
	Middles	0.50	0.43	0.44	0.51
	Bottoms	0.44	0.38	0.43	0.42
	Weighted Av.	0.50	0.42	0.43	0.50
% Total Ash	Tops	19.00	15.81	19.65	18.08
	Middles	20.97	21.06	21.20	19.08
	Bottoms	21.67	23.49	17.65	25.25
	Weighted Av.	20.53	20.03	19.81	20.32

RESULTS AND DISCUSSION

The crop data presented in Table 1 are the averages for the two years, 1954 and 1955. Each chemical treatment resulted in a significant decrease in sucker growth and a significant increase in yield. Neither the grade index nor the crop index differed significantly between treatments.

The chemical analytical results for the 1955 crop are given in Table 2. The weighted-average percentage values are the true averages of the various constituents in the total leaves per plant, allowing for the differential weights of the three grades. The maleic hydrazide treatments resulted in a decrease in the percentage content of total alkaloids, nicotine, calcium, and sulphur in the weighted average and in the three grades, with two exceptions—a slight increase in total alkaloids in the bottom leaves and a moderate increase in calcium in the top leaves of the higher MH-30 treatment. There was little change in the leaf content of nornicotine, total nitrogen, potassium, phosphorus, magnesium, and chlorine. The mineral oil treatment resulted in a reduction in total alkaloids, nicotine, and calcium in the three grades and the weighted average but had little influence on the nornicotine, total nitrogen, potassium, phosphorus, magnesium, and sulphur. An increase in chlorine in both the top leaves and the bottom leaves resulted in only a slight increase in the weighted average over the control. The data on total ash are somewhat irregular within the three leaf grades of each treatment; however, the average for each treatment is lower than that for the control.

It is evident that both the maleic hydrazide and the mineral oil treatments reduced sucker growth significantly without any deleterious effects on yield, grade index, and crop index. The changes in the chemical

composition of the cured leaves associated with the chemical treatments were not of a high order. The decrease in the total alkaloids and nicotine in the leaves may be regarded as favourable to the production of cigar tobacco of good quality.

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THE EFFECTS OF SEEDING RATE AND ROW WIDTH IN RELATION TO SEED PRODUCTION IN ORCHARD GRASS, *DACTYLIS GLOMERATA* L.[1]

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ABSTRACT

The effects of six seeding rates and five row spacings on seed yield, seed quality and the yield components—number of fertile culms per square foot, number of seeds per culm and seed weight were studied in orchard grass.

Row width had a marked effect on seed yield each year. Rate of seeding affected seed yield to a lesser extent and a row width x seeding rate interaction did occur. Seeding rates and row width caused small relatively unimportant differences in seedling establishment and early seedling vigour only in the third harvest year. Considering seed yield, seed quality and the ease of weed control, the best treatment combination appeared to be a seeding rate in the area of 7½ lb. per acre in 14-in. rows for stands to produce for 2 or 3 years.

Seed yield was positively and closely correlated with the number of fertile culms in the first 2 crop years but a negative relationship was found in the third crop year. Seed weight was negatively correlated with seed yield. The number of seeds per panicle was closely associated with yield in the third crop year and negatively correlated with seed weight and culm numbers.

INTRODUCTION

Canada imports approximately 600,000 pounds of orchard grass seed each year. The bulk of this seed is used in Ontario where the species is well adapted to the southern parts of the province. In Ontario, the wide range of climatic and soil conditions provides an area where the seed production of several grass species should be feasible and profitable. However, little effort has been made to check the performance of grasses as seed crops and establish suitable methods of seed production. The study reported herein was designed to determine the effect of rate of seeding and row width on seed yield and seed quality in orchard grass.

Grasses grown in rows have generally yielded more seed than when grown in solid stands (1, 4, 5, 6, 8). Exceptions to this have been noted, however, by some workers (2, 3, 6, 8). In a review of seed production in European countries, Schwanbon and Froier (7) reported orchard grass seed production was favoured by row widths of 18 to 24 inches.

Klages and Stark (6) found bromegrass seed yields were depressed when seeding rates exceeded 4 pounds per acre but rates of seeding had little effect on orchard grass, crested wheatgrass and meadow fescue. Spencer (8) found seed yields of Ky. 31 fescue and orchard grass decreased as the seeding rate increased from 3 to 7 to 15 pounds per acre. He also reported that the seed produced from rows was larger and heavier than seed produced from solid stands. Klages and Stark (6) found orchard grass produced a higher number of fertile culms when seeded at 4 pounds per acre than at higher seeding rates. With all species studied, the number

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of fertile culms was higher in row than in solid seedings. These numbers decreased with the age of stand, but the decrease was more pronounced in solid seedings.

MATERIALS AND METHODS

The experiment was conducted on Peel clay-loam soil of medium-low fertility with a pH of 7.0 on the College farm at Brampton. Fertilizer was applied at 300 pounds of 4-24-12 per acre worked into the soil in May and 200 pounds of 4-12-10 drilled prior to seeding in August. Annual applications of 150 pounds of ammonium nitrate were made by mid-April in each producing year.

Oron orchard grass was seeded with a small plot seeder on August 20, 1954. Excellent growth was obtained before freeze-up. Seeding rates of 2½, 5, 7½, 10, 12½ and 15 pounds of seed per acre were used, each in combination with row widths of 7, 14, 21, 28 and 35 inches in a split-plot design with four replications. Row widths constituted the main plots and rates of seeding the sub-plots. The plots were 30 feet long and contained the following number of rows seeded and harvested:

Row width	Number rows seeded	Number rows harvested
7 inch	18	10
14 inch	8	6
21 inch	6	4
28 inch	5	3
35 inch	4	2

Plots with row widths of 21 inches or greater were cultivated in the spring and fall to control weeds. Four counts per plot of 1 foot of row were converted for fertile culm number determinations per square foot, 50 panicles per plot for number of seeds per panicle. In order to assess seed quality, measurements were taken on seed weight, percentage establishment and seedling vigour. Seed weight was determined by weighing 200 seeds per plot. To determine establishment, 100 seeds per treatment were seeded at a depth of ½ inches in a 4-inch clay pot in the greenhouse. Average seedling height at 40 days after seeding was used as a measure of seedling vigour.

RESULTS AND DISCUSSION

Seed Yields

The seed yields obtained in this study are given in Table 1. Rate of seeding, in general, had less effect on seed yield than did row width. In 1955, and on the average over the 3 years, however, a significant rate x row width interaction was found. Over the 3-year period highest seed yields were obtained from rates of 7½ to 15 pounds in the 7-inch rows, 7½ to 10 pounds in the 14-inch rows, 5 pounds or above in the 21-inch rows, and 2½ pounds in the 28- and 35-inch rows. These differences were largely due to the yield differences obtained in 1955. In that year, the only year

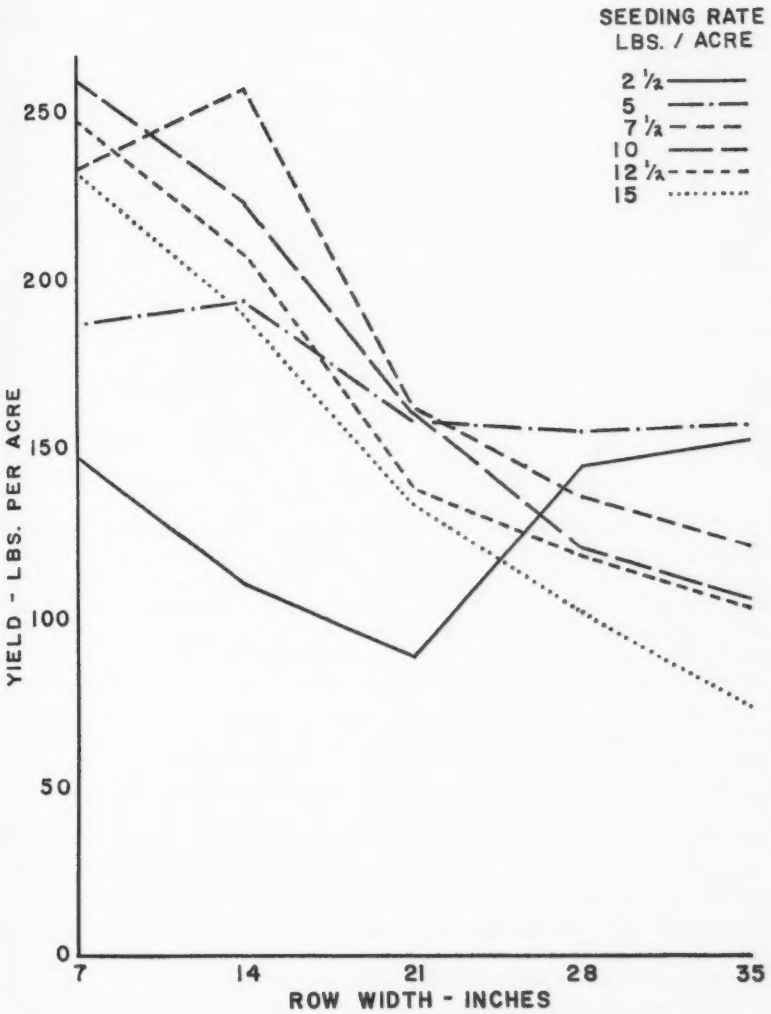

FIGURE 1.—Effect of seeding rate and row width on the yield of seed per acre in 1955.

in which a row width x seeding rate interaction was found, row width had more effect on yield where light seeding rates ($2\frac{1}{2}$ and 5 pounds) were used (see Figure 1). The $2\frac{1}{2}$-pound rate yielded considerably less seed than the other rates until the row width reached 28 inches. The most satisfactory yield was obtained from the seeding rate in the area of $7\frac{1}{2}$ pounds in the 14-inch rows.

TABLE 1.—SEED YIELD IN POUNDS PER ACRE

Rate of seeding lb./acre	Row width					Rate mean			
	7″	14″	21″	28″	35″	3-year av.	1955	1956	1957
2½	161	169	180	239	228	196	128	293	164
5	165	209	209	218	208	202	169	292	144
7½	190	225	207	213	197	206	181	297	141
10	197	223	209	223	196	210	173	299	157
12½	198	200	206	218	191	202	163	302	143
15	195	200	203	204	191	199	146	301	149
Row width mean									
3-year av.	184	204	203	219	202	202			
1955	216	196	140	129	119		160		
1956	273	324	318	301	270			297	
1957	64	93	150	226	217				150

L.S.D. (0.05)	Rates	Row widths	Rates x row widths	C.V.
3-year av.	9	20	19	6.7
1955	28	31	Sig.	27.3
1956	N.S.	40	N.S.	9.8
1957	17	39	N.S.	17.6

A rate x year interaction is evident in Table 1. The 2½-pound rate, compared to most higher rates, produced the lowest yields in 1955, similar yields in 1956, and higher yields in 1957. This indicates that lower rates might be superior for seed fields which are to be maintained in production for several years, whereas medium rates might be superior for short-term seed fields especially those down for one harvest year only.

Row widths caused seed yield differences each year. In the first crop year seed yields decreased as row widths increased. In the second crop year the intermediate row widths were superior. In third year of production seed yields increased markedly as the row width increased up to 28 inches. On the average, the 7-inch row width was inferior to the other widths and never yielded more than the 14-inch rows. The 14-inch row width had the advantage over wider row widths of not requiring cultivation for weed control. Throughout the study, this row width provided sufficient competition to eliminate weeds and produce a weed-free crop. The combination of a seeding rate approximating 7½ pounds in 14-inch rows was not surpassed in the first 2 years and on the average over the 3-year period, although this was not the highest yielding combination in the third crop year.

The average seed yield obtained was satisfactory. Yearly differences, however, did occur. The lower yields obtained in the first crop year might be attributed to the previous August seeding with a consequent lack of adequate plant development. The lower yields in the third year suggests a "sod bound" condition since the light rate of seeding did not depress yields to the extent that the heavier rates did, and the 28- and 35-inch row widths produced heavier yields than the narrower widths. It appears that seedings made for long-term seed producing stands (more than 3 years) should involve a low rate of seeding in rows at least 28 inches apart.

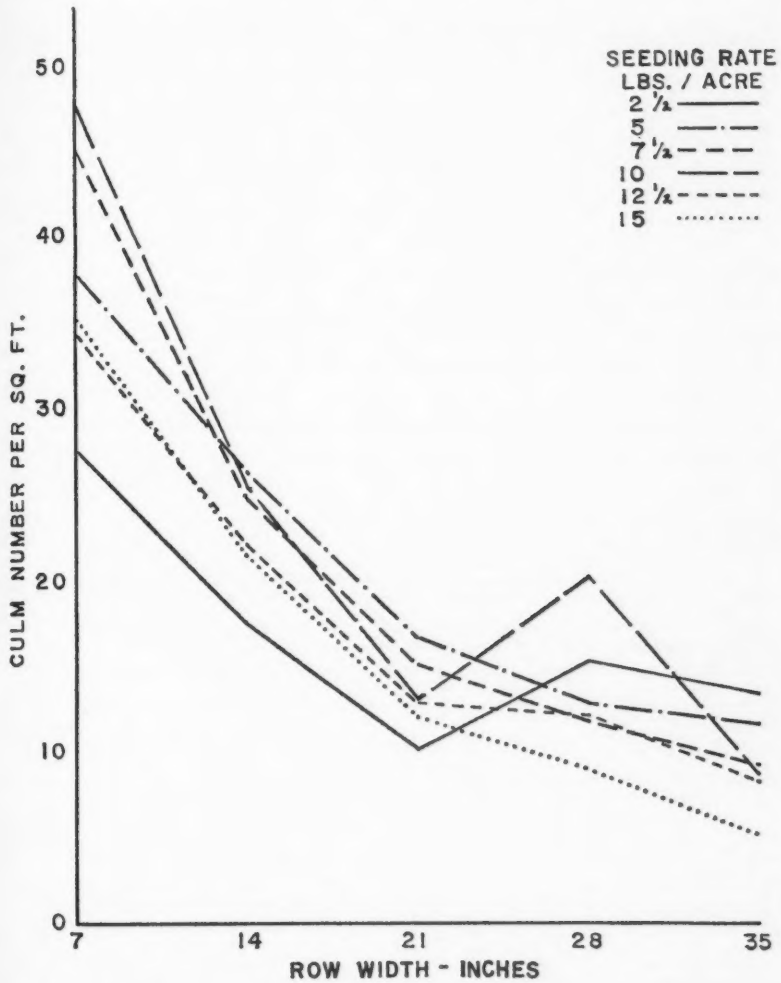

FIGURE 2.—Effect of seeding rate and row width on the number of fertile culms per square foot in 1955.

Fertile Culms

Variation in rates of seeding had less effect on fertile culm numbers than did variation in row widths (Table 2). On the average of the 3 years, rates up to 7½ pounds produced more fertile culms than did higher rates. An interaction of rates x row widths was present in 1955 but not in the other years or the 3-year average. Figure 2 shows that this interaction was caused in part by larger differences among seeding rates at the 7-inch row width than at other row widths. It was also partially caused by the 2½-

TABLE 2.—NUMBER OF FERTILE CLUMS PER SQUARE FOOT

Rate of seeding lb./acre	Row width					Rate mean			
	7″	14″	21″	28″	35″	*3-year av.	1955	*1956	1957
2½	37.4	37.8	31.9	27.2	23.9	31.6	16.7	25.6	42.7
5	40.0	41.1	35.8	25.7	20.4	32.6	21.0	26.0	41.7
7½	42.5	41.1	34.1	25.2	21.1	32.8	21.2	26.6	39.3
10	42.5	37.8	26.5	24.1	21.2	30.4	21.0	25.5	38.6
12½	38.3	39.1	29.6	24.6	22.3	30.8	17.8	26.4	39.8
15	37.7	37.7	30.3	24.4	19.9	30.0	17.0	26.5	38.2
Row width mean									
*3-year av.	39.7	39.1	31.4	25.2	21.5	31.4			
1955	38.4	22.7	13.3	11.8	9.3		19.1		
*1956	28.1	38.3	32.5	20.3	18.4			26.1	
1957	52.7	43.8	37.4	36.0	30.2				40.0

L.S.D. (0.05)	Rates	Row widths	Rates x row widths	C.V.
3-year av.	1.7	3.6	N.S.	7.5
1955	2.8	2.2	Sig.	23.7
1956	N.S.	8.7	N.S.	11.1
1957	3.2	5.2	N.S.	12.8

* 3 replications

TABLE 3.—NUMBER OF SEEDS PER PANICLE AND 1000-SEED WEIGHT

Rate of seeding lb./acre	Number seeds per panicle			1000-seed weight in mg.			
	1956	1957	2-year av.	1955	1956	1957	3-year av.
2½	269	95	175	125.3	105.8	125.0	118.7
5	257	97	170	124.5	105.4	126.1	118.7
7½	235	84	153	124.4	105.6	128.1	119.3
10	251	89	165	122.4	106.7	126.2	118.5
12½	260	85	167	122.9	110.1	125.9	119.6
15	257	90	170	121.1	109.9	126.2	119.0
Row width							
7″	217	51	127	116.1	107.4	126.0	116.5
14″	233	54	137	121.0	105.2	127.5	117.9
21″	275	81	168	125.7	105.5	129.9	120.3
28″	271	120	190	127.4	108.4	124.4	120.1
35″	278	147	210	127.1	109.9	123.5	120.2
General mean	255	90	167	123.4	107.3	126.2	119.0

L.S.D. (0.05)	Rates	Row widths	Rates x row widths	C.V.
Number of seeds per panicle 1957	N.S.	19	N.S.	18.3
1000-seed weight 1955	26	54	N.S.	3.7
1956	36	N.S.	N.S.	5.3
1957	N.S.	N.S.	N.S.	2.3
3-year average	N.S.	16	N.S.	2.1

pound rate producing the lowest number of fertile culms at row widths of 21 inches or less and the highest number at the 28-inch (10-pound rate exception) and 35-inch row widths.

Fertile culm numbers increased each year of the study. A rate x year interaction is evident because the 2½-pound rate increased more from 1955 through 1957 than did other rates, especially the intermediate rates. For example, the 2½-pound rate was lower than the 5-pound rate in 1955, the same in 1956, and higher than all other rates in 1957.

On the average, the fertile culm numbers decreased in a linear manner as the row width increased (Table 4). In each year, there were differences in culm numbers due to row widths. The degree of difference, however, was greatest in 1955 where four times as many fertile culms were produced in the 7-inch rows as in the 35-inch rows.

Number of Seeds per Panicle

Table 3 shows seeding rates did not affect the number of seeds per panicle in 1957. The interaction between seeding rates and row widths also was not significant.

TABLE 4.—"F" VALUES FOR THE LINEAR AND QUADRATIC COMPONENTS OF THE VARIANCE FOR ROW WIDTH OVER THE THREE-YEAR PERIOD

	Degrees of freedom	Seed yield	Culm numbers	Weight of 1000 seeds	Per cent establishment	Seedling height
Row widths	4	3.47*	54.79**	3.66*	1.62	0.24
Linear	1	5.69*	209.51**	11.26**	0.01	0.35
Quadratic	1	5.03*	1.98	2.50	4.66	0.02
Deviation from reg.	2	1.58	3.84	0.44	0.80	0.30

TABLE 5.—ESTABLISHMENT AND SEEDLING VIGOUR, THREE-YEAR AVERAGE

Rates of seeding	2½	5	7½	10	12½	15
Establishment (%)	78.1	77.3	77.6	77.3	78.1	78.6
Seedling height (cm.)	9.9	10.1	10.1	10.0	10.0	10.0

Row widths	7″	14″	21″	28″	35″
Establishment (%)	76.9	77.8	80.3	77.7	76.4
Seedling height (cm.)	9.9	10.0	10.1	9.9	10.2

In 1957, wide differences were present among the row widths and differences, although smaller, also would appear to be present in 1956. In both years, the number of seeds per panicle increased as the row width increased. Differences among row widths were more pronounced in the third harvest year than in the second. In the third year, 65 per cent fewer seeds were produced per panicle.

Seed Weight

The rates of seeding affected the seed weight in 1955 and 1956 (Table 3). In the first harvest year, the light seeding rates produced heavier seed than did the 10- to 15-pound rates. In 1956, however, the order was reversed with the heaviest seed produced from the 12½- to 15-pound rates. Seeding rates had no effect on seed weight in 1957.

In 1955 and on the 3-year average, row widths affected the seed weight. In general, seed weight increased in a linear manner as the row width increased (Table 4). The effect of row width was most pronounced in 1955.

Establishment and Vigour

Table 5 shows the average establishment and seedling height obtained in this study. Significant differences were obtained in 1957 only. In that year, small but relatively unimportant differences in the number of plants established and the vigour of the seedlings were detected.

Interrelationship of Characters

The coefficient of correlation values in Table 6 show that the number of fertile culms was closely associated with seed yield in each year of the study. In 1955 and 1956, the relationship was positive indicating that an increase in culm number resulted in a yield increase. In the third crop year the average number of culms was 40.0, an increase over the previous year of 100 per cent. This culm number of 40 appears to exceed the optimum in that a high negative correlation was obtained between seed yield and number of culms in the third crop year. Inspection of the data from individual years in Tables 1 and 2 indicates that the row widths with the highest seed yields in each year had a stand density of 36 to 38 culms per square foot. Even in 1957, when the highest rate had an average of 42.7 culms,

TABLE 6.—CORRELATION OF PLANT CHARACTERS

Correlation of:	1955	1956	1957
Culm number—seed yield	+.827**	+.769**	−.825**
1000-seed weight—seed yield	−.639**	−.267	−.389*
Number of seeds per panicle—seed yield	—	+.049	+.907**
Number of seeds per panicle—1000-seed weight	—	+.318	−.529**
Number of seeds per panicle—culm number	—	−.164	−.774**
1000-seed weight—culm number	−.791**	−.402*	+.179
1000-seed weight—establishment	−.256	−.0008	+.260
1000-seed weight—seedling height	−.287	+.125	+.025
Necessary r .361 (0.05); .463 (0.01)			

inspection of the data indicates that the 28- and 35-inch rows were mainly responsible for this higher average yield and these had 37.0 and 32.4 culms per unit area, respectively. The higher yielding treatment was the former.

Seed weight was negatively associated with seed yield each year, although in 1956 the correlation coefficient was not significant. This negative association does not appear to be an important consideration in that it was not reflected in either establishment or seedling vigour. Even in 1957, when small differences were detected in establishment and seedling vigour, the correlation coefficients of seed weight with these characters were not significant.

Correlations of the number of seeds per panicle with other characters are available for 1956 and 1957 only. In 1956 the number of seeds was not related to seed yield but a highly significant relationship was found in 1957. This suggests that the number of seeds per panicle becomes important as a factor determining yield when the optimum or an excess number of fertile culms is present.

Considering the three yield components—numbers of culms, number of seeds per culm and seed weight, it appears that seed weight although negatively associated with yield was not as important in determining yield as were the other two components. Number of culms was the dominant component in 1956 and in that year number of seeds was not a differentiating factor. In 1957 both number of culms and number of seeds per panicle had important effects on yield. Row widths with highest seed yields had culm numbers between 30.2 and 37.4. Culm numbers higher than this were associated with lower yields. There were greater differences in number of seeds per culm in 1957 than in 1956 and this may have been responsible for the correlation of seed yield with number of seeds being high only in 1957.

Seed weight decreased as culm numbers increased in the first two crop years, but these characters were not related in the third crop year. Number of seeds per panicle was not related to culm number in 1956 but was in 1957.

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EMBRYO-SAC PRODUCTION IN RELATION TO SEED YIELDS OF DIPLOID DOLLARD RED CLOVER[1]

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ABSTRACT

Evidence that red clover plants of the Dollard variety differ in production of fertile ovaries is presented. The amount of ovary sterility arising through failure of the embryo sac to complete its development is apparently controlled by genetic factors and influenced by environmental conditions. Consideration of the data on frequencies of ovary fertility of plants in nursery lines and in progenies of crosses, together with data on their seed production, leads to the conclusion that ovary sterility is an important limiting factor on the ability of a plant to produce seed. Even under adverse environmental conditions, appreciable ovary sterility is still the primary limiting factor in restricting seed production and hence, among plants affected by ovary sterility, it is not surprising that we found significant correlation between the percentages of ovary fertility and seed yields.

INTRODUCTION

Recent studies (8) on fertility in diploid red clover of the Dollard variety led the authors to conclude that meiotic irregularities were too few to account for the high frequencies of abnormal pollen grains produced by many plants. Also, the amount of pollen abortion was not correlated significantly with the amount of seed set, so that partial male sterility did not seem to be a controlling factor in the setting of seed. There was some evidence, however, that failure of embryo-sac production in certain plants is one cause of low seed set and that no obvious relationship exists between the extent of pollen abortion and the ability of a plant to produce apparently normal embryo sacs. Accordingly, it was decided to investigate the ability of plants to produce embryo sacs as a possible factor of importance in the setting of seed.

Aside from Martin's description of embryo-sac development in red clover (6), there is very little information available on this subject. He found 1-4 archesporial cells and a longitudinal row of four megaspores of which the lower one functions to produce an 8-nucleate embryo sac in which the three ephemeral antipodals have completely degenerated with no trace remaining at fertilization time. Thus the mature embryo sac contains an egg, two synergids and a polar fusion nucleus. Very similar development of the embryo sac has been described by Cooper for *Melilotus* (2) and for *Medicago sativa* (3) and by Hansen for *Lotus corniculatus* (5). Das (4) found that in both high- and low-fertility lines of *Melilotus alba* most ovules develop normal embryo sacs and that a relationship exists between the frequency of production of abnormal embryo sacs and the extent of sterility.

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MATERIALS AND METHODS

All plants used were of the double-cut variety Dollard of diploid red clover and selected from various lines growing in the Dollard breeding nursery of the Agronomy Department at Macdonald College. Florets for embryo-sac studies were fixed in CRAF fluid at anthesis or 2 days after the florets had opened. The ovaries were dissected out under a stereoscopic microscope, embedded in paraffin, sectioned at 15 μ and stained in Heidenhain's iron hematoxylin.

An ovule was classified as containing an embryo sac when an egg, two synergids, and one polar fusion nucleus (or two polar nuclei) were present in the micropylar end of the nucellar cavity, or at some distance from it (Figure 7). An ovule was considered as abortive or containing no embryo sac when the whole nucellus had apparently degenerated, i.e., showed not even a residue of the embryo sac (Figure 9); or when some other abnormality was present at the time of fixation, such as the presence of only one or two nuclei (Figures 2, 5), the absence of synergids (Figure 8), the signs of development of a secondary embryo sac at the chalazal end of the nucellus (Figure 3); or when the embryo sac was in the process of degenerating or being absorbed (Figure 6).

Seed set was determined as either the number of seeds per head under open pollination conditions, *or* the percentage of florets setting seed under open pollination conditions, *or* the percentage of florets setting seed after crossing.

In order to determine the percentage of good pollen, 3 to 4 florets were tripped, the pollen was placed on a slide in a drop of lactophenol to which a little fast green stain had been added to stain the contents of apparently normal pollen grains, and a sample of about 600 pollen grains was examined for each plant.

RESULTS OBTAINED

Number of Ovules per Ovary

Martin (6) found only two ovules per ovary as shown in Figures 1 and 9; however, exceptions do occur (Figure 4.) In a limited population of 100 plants in 1954-56, the numbers of ovules per ovary were determined and are shown in Table I.

TABLE 1.—OVULES PER OVARY IN A LIMITED POPULATION OF DIPLOID DOLLARD RED CLOVER

Number of ovules per ovary	Numbers of ovaries	Percentages
1	3	0.10
2	2913	99.05
3	24	0.82
4	1	0.03
Total	2941	100.00

TABLE 2.—EMBRYO-SAC PRODUCTION AND SEED SET IN TWELVE RED CLOVER PLANTS

Plant No.	Yield type	Production of mature embryo sacs, 1954					Florets setting seed (%)	No. of seeds per head O.P.[2]
		No. of ovaries examined	Ovary types (%)[1]			Ovary fertility (%) (01+11)	1954	1953
			00	01	11			
1	Low	114	96.5	2.6	0.9	3.5	2.8	3.7
2	Low	119	65.5	28.6	5.8	34.4	12.6	20.8
3	Low	99	55.6	33.3	11.1	44.4	0.6	0.3
4	Low	131	48.9	42.7	8.4	51.1	6.4	8.3
5	Low	108	30.5	43.5	26.0	69.5	20.1	18.0
6	Low	105	19.0	50.5	30.5	81.0	4.6	5.4
7	High	106	23.6	53.8	22.6	76.4	24.0	54.0
8	High	100	15.0	36.0	49.0	85.0	76.1	64.2
9	High	125	10.4	19.2	70.4	89.6	91.7	95.7
10	High	126	9.6	45.2	45.2	90.4	93.1	91.7
11	High	109	1.9	22.9	75.2	98.1	75.0	83.2
12	High	109	0.0	11.9	88.1	100.0	51.6	109.5
Means	Low		52.7	33.5	13.8	47.3	7.8	9.4
	High		10.1	31.5	58.4	89.9	68.6	83.0

[1] 00—neither of the two ovules in an ovary contained an embryo sac,
01—one ovule contained an embryo sac and the other did not,
11—both ovules contained embryo sacs.

[2] O.P.—open pollination.

In one plant 8 ovaries with three ovules each were found in a total of 23 ovaries examined. In this plant and in most other instances the ovules in excess of two per ovary stopped at some early stage of development, or they were slower in development than the others. These results support the general view that the ovary of red clover regularly contains two ovules.

Embryo-sac Production in Selected Plants

On the basis of the number of seeds per head, produced under open pollination (O.P.) in the field in 1953, six plants were selected as low yielding and six as high yielding. In 1954 the production of embryo sacs in a large sample of ovaries for each plant was examined and each ovary classified as 00 where neither of the two ovules contained a mature embryo

PLATE I. Photomicrographs of ovaries and ovules of diploid red clover at early stages of development.

FIGURE 1. Longitudinal section of a normal floret with two levels of anthers at the pollen grain stage, and two ovules with meiosis stage in the megaspore mother cells. (X45).

FIGURE 2. Abnormal condition of the embryo sac at an early stage. (X120).

FIGURE 3. Development of the embryo sac in the chalazal end of the aborting nucellus. (X120).

FIGURE 4. Ovary containing three ovules; the development of the lowermost is defective (outer integument not formed, embryo sac irregular). (X45).

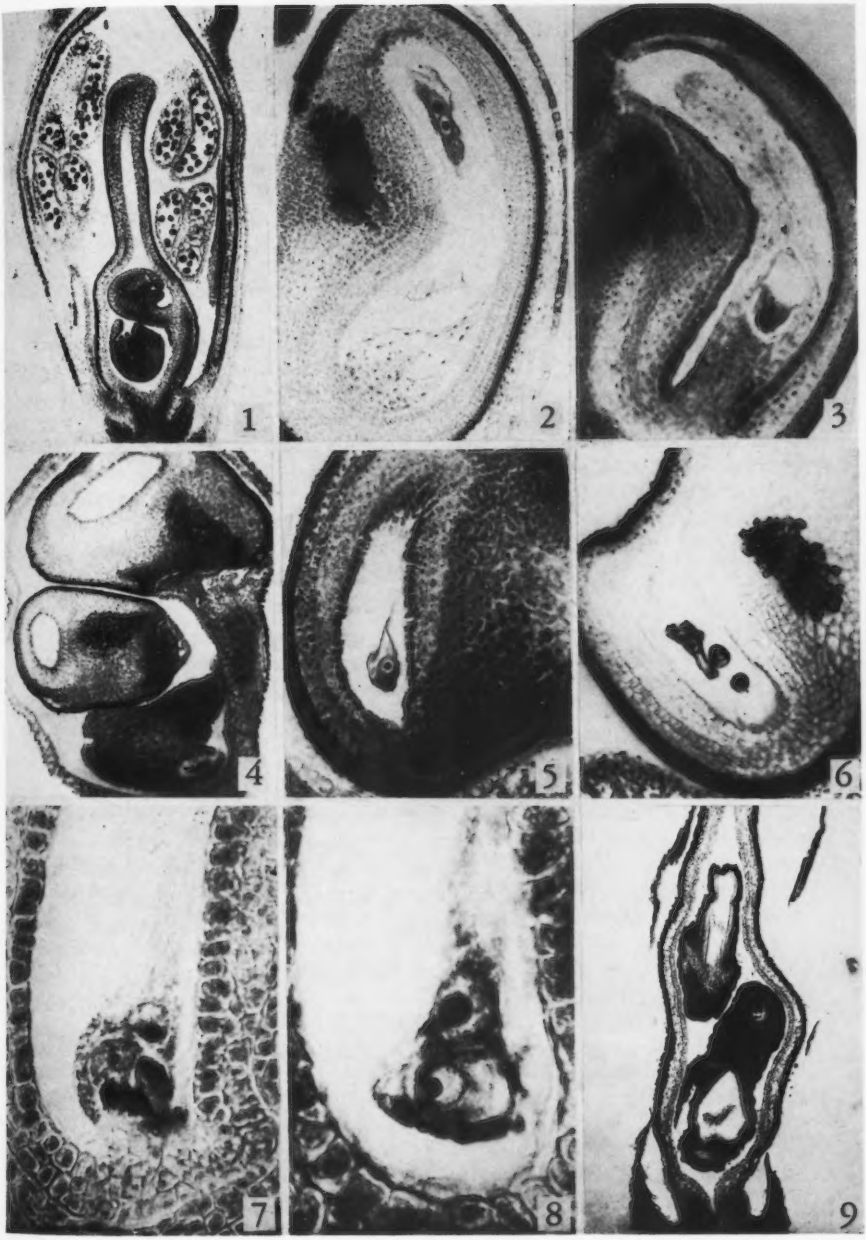
FIGURE 5. Abnormal condition of an embryo sac at an early stage. (X120).

FIGURE 6. Elements of an aborting embryo sac still persisting in an ovule. (X120)

FIGURE 7. A normal egg apparatus consisting of an egg, polar fusion nucleus and two synergids. (X390).

FIGURE 8. An abnormal egg apparatus (no synergids). (X650).

FIGURE 9. A shrunken ovary with both ovules aborted. (X60).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sac, as 01 where only one of the two ovules contained such an embryo sac, and as 11 where both ovules contained such an embryo sac. As there is regularly only 1 ovary per floret, the percentage of potentially fertile ovaries was then calculated using the total of 01 and 11 types over the total number of ovaries examined. On each plant several heads, cross pollinated at the same time, were allowed to mature and scored for the percentage of florets setting seed. The results obtained are shown in Table 2.

It is clear from the data in Table 2 that most of the plants selected as low yielding in 1953 produced many ovaries of the 00 type and were low in ovary fertility and seed yield in 1954. On the other hand, most of the plants selected as high yielding in 1953 had few 00 type ovaries with a majority of the 11 type, plus high ovary fertility and good seed set in 1954. Plants 5 and 6 in the low-yielding category were exceptional in that they had high ovary fertility but still did not yield well, and plants 7 and 12 in the high category were exceptional in that their yields were lower than expected in 1954. All of these exceptions indicate that factors operating after the production of mature embryo sacs may also seriously affect seed production. The data still support the view that some plants yield little seed because of a shortage of mature embryo sacs available at the time of fertilization. Presumably the effects of a small change in percentage of ovary fertility may not be detectable when the seed is mature because other factors acting later in development can have a much stronger influence on seed yield.

Embryo-sac Production in Different Lines

Having shown the existence of variation in the production of embryo sacs between selected individual plants, efforts were made to explore the presence or absence of such variations between different populations. Ten lines of the Dollard variety (of which lines 5 and 6 were later shown to have come from the same source), grown under open pollination conditions, were available for this test in the polycross progeny nursery of the Agronomy Department in 1954. Fifteen plants per line were sampled and usually 10 ovaries per plant were sectioned, stained and examined. Twenty heads were examined at maturity to determine the number of seeds per head. Weather conditions during the summer 1954 were very unfavourable for fertilization and subsequent seed growth. These adverse conditions probably lowered the production of embryo sacs and the number of seeds per head. The data are summarized in Table 3.

There are obvious differences between lines in the frequencies of different types of ovaries. Although differences between adjacently recorded lines may not be statistically significant, as one progresses from line 1 to 10, there is shift from left to right, i.e., from a low frequency of the 00 type to a low frequency of the 11 type of ovary. This shift is reflected in a corresponding decrease in the means of percentages of fertile ovaries, as would be expected. The direct comparison of the line means of percentages of fertile ovaries using their standard errors shows that certain lines differed significantly from others though they were exposed to the same general environmental conditions. It appears that certain lines clearly produce a higher number of fertile ovaries than others and are, therefore, potentially higher seed yielders.

TABLE 3.—EMBRYO-SAC PRODUCTION AND SEED YIELD IN NINE LINES OF DOLLARD RED CLOVER, IN 1954

Line	No. of plants	Total ovaries examined	Av. ovary types (%)			Fertile ovaries (01+11) (%)				No. of seeds per head O.P.		Correlation coefficients[2]
			00	01	11	Means	Range of variation	Transformed into angular units				
								Means	SE[1]	Means	S.E.	
1	15	148	13.5	37.8	48.7	86.5	70–100	71.8	3.25	42.7	2.18	–0.061
2	15	148	13.5	37.2	49.3	86.5	60–100	73.1	3.96	33.7	3.70	0.195
3	15	140	20.7	43.6	35.7	79.3	60–100	66.4	3.58	45.1	2.58	0.345
4	13	123	27.6	44.8	27.6	72.4	40– 90	59.5	3.01	44.0	3.51	0.634*
5	15	144	28.3	40.3	31.4	71.7	10–100	61.5	5.28	27.4	2.23	0.569*
6	15	147	32.7	34.0	33.3	67.3	0–100	56.6	5.74	33.1	3.72	0.705**
7	15	147	34.0	39.5	26.5	66.0	20–100	55.8	3.50	26.5	4.43	0.201
8	15	145	37.2	33.8	29.0	62.8	0–100	54.7	6.41	31.2	3.16	0.522*
9	15	149	40.3	36.2	23.5	59.7	10– 90	50.8	3.32	28.7	4.58	0.350
10	15	142	45.8	40.1	14.1	54.2	11–100	47.5	3.90	38.9	3.45	0.308
Av.	148	1,433	29.4	38.7	31.9	70.6		59.8		35.1		

[1]S.E. = Standard Error
[2]Correlation Coefficient between percentage of fertile ovaries and number of seeds per head
*Significant at the 0.05 level
**Significant at the 0.01 level

TABLE 4.—COMPARISON OF GROUPS OF RED CLOVER PLANTS SET UP ON THE BASIS OF OVARY FERTILITY

Group	No. of plants	Ovary fertility %		No. seeds per head O.P.		r between % fertile ovaries and no. seeds
		Range	Mean	Range	Mean	
1	50	0.0– 66.6	44.87	0.3–52.8	27.63	0.387**
2	48	66.7– 87.0	74.41	0.2–68.1	36.02	−0.177
3	50	87.1–100.0	93.71	13.0–88.1	41.30	−0.055
All plants	148	0.0–100.0	70.95	0.2–88.1	34.97	0.393***

**Significant at the 0.01 level.
***Significant at the 0.001 level.

Apparently certain lines are more variable in respect to ovary fertility than are others as evidenced by the larger ranges of variation. For example the range of variation of line 1 is from 70 to 100 per cent whereas that for line 6 is from 0 to 100 per cent. Also, apparently the lines that produce the higher frequencies of fertile ovaries are less variable in this respect since most of their ranges of variation are narrower than the ranges of variation of the lines producing lower frequencies of fertile ovaries. If one assumes that environmental variables have an equal chance of operating at random within all of these lines then these differences in the ranges of variation plus the differences between means suggest the existence of more genetic variation in lines that produce fewer fertile ovaries and perhaps also in those producing less seed.

From the examination of the correlation coefficients between the percentages of fertile ovaries and of numbers of seeds per head, for plants within each line, it is notable that they were significant in only four lines. The same correlation coefficient for the pooled population of all lines is 0.393 and significant at the 0.001 level (see Table 4). Apparently the adverse weather conditions and the low numbers of plants masked the correlations within most of these lines. However, plants producing few fertile ovaries cannot possibly produce much seed and also other factors can operate more strongly when there are abundant fertile ovaries available. Accordingly we decided to compare plants producing few fertile ovaries with those producing many fertile ovaries and this led to the analysis shown in Table 4.

As seen in Table 4, we divided the pooled population of the ten lines into three nearly equal groups on the basis of the ability of each plant to produce fertile ovaries. Proceeding from Group 1 to 3, as the mean values for the percentages of fertile ovaries increase, the mean values for numbers of seeds per head also increase somewhat. Even more interesting is the significant positive correlation (0.01 level) between the percentages of fertile ovaries and numbers of seeds per head for plants of Group 1 and the absence of such significance in Groups 2 and 3. Apparently the significance of this correlation for the general population is largely determined

by the strong correlation existing in Group 1. These data clearly show that many plants produce high percentages of sterile ovaries and suggest that such ovary sterility is a primary factor of great importance in limiting seed production of red clover. On the other hand, plants producing few sterile ovaries are not necessarily good producers of seed.

Embryo-sac Production in Progenies of Known Parentage

We made a preliminary check for genetic segregation in the production of mature embryo sacs using samples of two progenies of known parentage. Four parent plants were selected on the basis of seed yields under open pollination in 1953 and over 100 ovaries of each were examined for embryo sacs in 1954. Two of the plants, selected as high in ovary fertility (see Table 2, plant 11 as ♀ and plant 9 as ♂ parent), were crossed to obtain progeny A, and progeny B was obtained by crossing the third plant (Table 2, plant 10), high in such fertility, with the fourth (Table 2, plant 4) which was low. Samples of progenies A and B were planted in 1955 and of A again in 1956. Over 20 ovaries were examined for production of embryo sacs for each progeny plant and five heads per plant were examined at maturity for seed production. The parents were not available for planting with their progenies and accordingly data for them cannot be strictly compared with those obtained for the progeny samples. The data are presented together, however, in Table 5.

Both parents of cross A were high in ovary fertility and produced over 70 per cent of the 11 type of ovary. The plants of the progeny samples were nearly as good on the average in percentage of fertile ovaries and only six were below 75 per cent. (Five of these six should have been above 75 per cent on the basis of the percentages of florets setting seed). Thus the progeny of cross A were uniformly good in both years in their ability to produce mature embryo sacs and the single real exception could have resulted from very adverse environmental circumstances. Very few of them produced quite as many 11 ovaries as their parents but this does not seem to have been a serious handicap in seed production. One parent in cross B was high in ovary fertility but produced fewer 11 ovaries than parents of cross A; the other parent was poor in both of these categories. The plants of progeny B were highly variable in these and in other respects. The standard error (3.76) of this progeny sample with respect to percentage of fertile ovaries is directly comparable with that (2.09) for progeny A in 1955 grown in the same plot. In view of its much greater magnitude in the absence of a known environmental variable, and since genetic segregation might be expected to show up in progeny B, it seems reasonable to suggest that genetic factors affecting production of fertile ovaries (i.e. causing ovule sterility through restriction of production of mature fertile embryo sacs) are probably present and segregating in at least one parent of cross B. Of course, some of the variation is probably environmental in source and with such a small sample of ovaries per plant there is a fairly large sampling error, but even so, this suggestion seems reasonable.

In these progenies we also tried to correlate production of fertile ovaries with the proportion of normally staining and apparently normal pollen (good pollen). For progeny A the correlation coefficient was -0.081

in 1955 and +0.483 in 1956; the former not significant and the latter just significant at the 0.05 level. For progeny B it was −0.155 and not significant. Thus the data available suggested that ovary fertility and pollen fertility are independently determined in red clover plants.

As mentioned above, seed production was also observed in the samples of progenies A and B. Percentages of florets setting seed, of numbers of florets and of seeds per head were recorded. The correlation coefficient between percentages of fertile ovaries and the percentages of florets setting seed for progeny A in 1955 was +0.126 and not significant and in 1956 it was +0.823 and significant at the 0.001 level, whereas for progeny B in 1955 it was +0.329 and not significant. A possible explanation of the two low correlations is seen in the circumstance that the percentage of florets setting seed is higher than is possible from the percentage of fertile ovaries as determined for nine plants of progeny A in 1955 and for 14 plants of progeny B. There were only two such values in 1956 in progeny A. Such impossible values, probably resulting from the limited number of ovaries per plant that it was possible to examine, may tend to obscure such correlation as exists. Also in 1955 heads of progeny A were fixed for ovary studies between August 24 and September 13 and another set of heads was harvested October 13 to 24, whereas in 1956 the heads were fixed for ovary studies on September 16 and others on the same plants were harvested on October 5, so that the better synchronizing of fixation and harvesting dates may be at least partly responsible for the higher correlation in 1956. Even in the progeny B sample if one considers only plants producing less than 66.6 per cent fertile ovaries, as in Group 1 of Table 4, a significant correlation (+0.633, significant at the 0.05 level) is obtained with respect to these variables. These correlations, though they do not provide strong support for the claim that reduction of ovary fertility strongly affects seed yields, are consistent with this point of view. Perhaps one should also mention that seed yields of these two progenies support our earlier finding (7) that "by selecting high seed yielding parents and crossing them, one increases the probability of obtaining a progeny of high mean seed yield".

Embryo-sac Production under Different Environmental Conditions

Heads on each of four plants of progeny B were fixed for the determination of ovary fertility on two different dates, August 25 and September 10, 1955, and 25 ovaries were examined on each plant for both dates. The mean minimum temperature for four days before August 25 was 55.2°F. and the corresponding mean maximum temperature was 80.5°F. The corresponding mean minimum and maximum temperatures before September 10 were 48.7°F. and 77.3°F., respectively. The mean percentage of ovaries containing mature embryo sacs was 74.6 on August 25 and 92.7 on September 10, a difference significant at the 0.05 level. Unless there is a sampling error involved, it appears that the production of embryo sacs may be influenced by the prevailing temperature or by other environmental factors.

DISCUSSION AND CONCLUSIONS

The results reported above provide some basis for a preliminary consideration of the effect of the production of fertile ovaries on the seed-yielding capacity of red clover plants.

TABLE 5.—VARIATION IN OVARY CONDITION AND SEED PRODUCTION IN PROGENIES OF TWO CROSSES OF RED CLOVER

Cross	Plants	Production of embryo sacs					Production of seed O.P.	
		Total ovaries	Ovary types (%)			Ovary fert .(%)	No. seeds per head Means ± S.E.	% florets setting seed Means ± S.E.
			00	01	11	01+11 ± S.E.		
A	♀ parent, 1954	109	1.9	22.9	75.2	98.1 ——	83.2 (1953)	—— ——
	♂ parent, 1954	125	10.4	19.2	70.4	89.6 ——	95.7 (1953)	—— ——
	Progeny							
	25 in 1955	566	16.1	41.2	42.7	83.9 ± 2.09	104.3 ± 3.76	80.3 ± 1.31
	23 in 1956	607	12.2	36.7	51.1	87.8 ± 3.08	103.2 ± 4.16	74.3 ± 2.72
B	♀ parent, 1954	126	9.6	45.2	45.2	90.4 ——	91.7 (1953)	—— ——
	♂ parent, 1954	131	48.9	42.7	8.4	51.1 ——	8.3 (1953)	—— ——
	Progeny							
	29 in 1955	660	35.2	42.3	22.5	64.8 ± 3.76	96.1 ± 5.52	62.9 ± 3.21

Two ovules are regularly produced in each ovary, and only one ovary per floret in red clover plants. Very rarely three ovules may develop in one ovary and, only very rarely, two embryo sacs can be found in one ovule. In 54,000 mature florets of progenies A plus B observed during these studies we found only 19 that contained two seeds and never three. Thus, with rare exceptions, only one ovule per ovary becomes a mature seed and almost all others abort at some stage before maturity.

The production of one embryo sac ready for fertilization and capable of being fertilized is obviously a primary requirement for fertility in each ovary. If both ovules have mature functional embryo sacs, the ovary possesses a degree of insurance against sterility but in nearly all cases only one of the two ovules will mature into a seed. Thus ovaries of types 01 and 11 are potentially capable of producing seeds but the 00 ovaries are sterile. Hence any factor that acts on a plant so as to increase the frequency of the 00 type of ovary or to reduce the 01 type to the 00 type will contribute toward the sterility of that plant but the 11 type may be reduced to the 01 type without a reduction of fertility.

The results presented above contain clear evidence that plants vary in their production of fertile ovaries of both the 01 and the 11 types. Some plants produce high frequencies of sterile 00 ovaries and cannot possibly produce many seeds for this reason alone. Even if our sample of ovaries examined per plant is too small to provide an accurate measure of the overall ability of the plant to produce fertile ovaries, there can still be little doubt that certain plants are partly sterile because they do not produce enough fertile ovaries. It is equally clear that some plants have a high potential fertility because they produce many fertile ovaries.

Martin (6) stated: "The sterility of ovules is a prominent feature in *Trifolium pratense*". He reported that synapsis is absent in mother cells of sterile ovules in which "all the cells of the nucellus remain vegetative and hence no embryo sacs are found". He noted that ovule sterility seems to be favoured by moisture, and that this tendency to produce sterile ovules varies among plants under similar conditions and "suggests that it may be partly eliminated by selection". He further points out that this tendency to produce sterile ovules "no doubt, always lowers the percentage of seed production, and in some cases reduces it to almost zero". We have no positive evidence regarding his claim that wet weather increases sterility but we do believe that other types of ovule sterility are also present in our plants.

Though we have some evidence indicating that environmental factors can affect the production of embryo sacs, i.e. ovary fertility, it seems to us that genetic factors producing embryo-sac and ovary sterility are operating in some red clover plants. These factors appear to function by affecting adversely, either directly or indirectly, the development of the embryo sacs. Since in one cross between a plant of high ovary fertility with one of low ovary fertility we obtained a highly variable progeny in this respect with a very wide range of fairly uniform distribution, we are inclined to think that at least several pairs of alleles (perhaps polygenes) are involved but with the large sampling error involved in the determination of ovary fertility this remains only a suggestion. A detailed cytological study of megasporogenesis and embryo-sacs development such as that by Das (4)

under controlled environmental conditions, combined with genetic analyses of segregation, might clarify the relationship between the hereditary and environmental factors affecting the ability of red clover plants to produce embryo sacs.

Our plants were space planted and this may have contributed also to the environmentally conditioned variability of the characters studied, since Bartley and Weber (1) for soybeans found: "Heritability values for seed yield were consistently low in F_2 and F_3 spaced-plant populations and moderately large among F_3 progenies", and the latter were grown in progeny rows.

We have presented evidence that different lines, and families from particular crosses, differ in their production of mature embryo sacs apparently ready for fertilization (i.e. apparently fertile). In such lines and families, plants that produce few fertile ovaries will, of necessity, produce reduced quantities of seed but, of course, the seed yield of the line will of necessity depend upon the frequency of such plants and on other genetic and environmental factors that may act during later development of the ovule into a seed. It is to be expected also that in such populations the extent of ovary sterility in various plants will be correlated with low seed yield because it is the initial limiting factor on seed yield and sets the initial seed-yielding potential of the plant. Hence, in populations where many plants have low ovary fertility, we can expect to obtain a high correlation between ovary fertility and seed yield even under adverse environmental conditions because the main level of a plant's fertility is determined largely by its ovary fertility and other factors can act only secondarily and then only in determining the extent of further reduction of seed yield. On the other hand, where the plants of a population have high ovary fertility, genetic and environmental factors will act on later development more strongly so that adverse environmental conditions during these stages are likely to obscure the correlation between ovary fertility and seed yields. Under optimum environmental and genetic conditions of seed development the correlation may again be demonstrable because ovary fertility variation can then be the major determining factor acting on the seed yield. In general our observations support this thesis.

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CYTOGENETIC STUDIES IN THE GENUS *HORDEUM*

II. INTERSPECIFIC AND INTERGENERIC CROSSES[1]

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ABSTRACT

The relationships of species were studied by making crosses in the genus *Hordeum*. Most of the crosses were between *H. vulgare* and one of the wild species. Embryo culture was necessary to produce most hybrids. The methods used for the preparation of the nutrient agar and for the excision of the embryos are given. Data on the seeds harvested, embryos transplanted, embryos germinated, and on the number of surviving plants are listed for 111 interspecific and six intergeneric crosses. In most of these crosses viable embryos were produced which later germinated and grew normally for a time but later died. Fourteen viable hybrids were obtained.

INTRODUCTION

Studies in the genus *Hordeum*, involving interspecific crosses and crosses with related genera, have been in progress since 1951 by the Barley and Cytogenetics Units of the Cereal Crops Division, Ottawa. Two reports have been published (5, 8). This paper deals with the various crosses that have been attempted and the successes achieved, emphasizing particularly those hybrids in which embryos were excised and germinated on nutrient agar. Most of the crosses discussed involve cultivated barley, *H. vulgare*, in combination with one of the wild *Hordeum* species.

MATERIALS AND METHODS

The correctness of some of the species names referred to in this paper, as applied to the plants used, is limited as explained in a previous publication (8). Therefore all stocks have been maintained under a "B" number assigned by the Cereal Crops Division. Species listed in this paper will be identified by this number. The numbers or variety names appearing after *H. vulgare* indicate the specific lines or varieties being used at this station. The letters "I.H." refer to interspecific hybrids produced in this program.

The nutrient agar was prepared by mixing nutrient solutions with liquid agar. The procedure, with some modifications, is that proposed by Randolph and Cox (9) and Konzak *et al.* (6). A few of the details are outlined here. A number of different types of agars were tried but "Difco Bacto-Agar Standardized" was found to be the most suitable. The nutrient solutions were kept as stock solutions and were prepared as follows:

Solution A		Solution B		Solution C	
Water	500 ml.	Water	250 ml.	Water	250 ml.
$Ca(NO_3)_2.H_2O$	35 g.	$Fe\ SO_4.7H_2O$	0.2 g.	$(Na\ PO_3)n$	1.5 g.
KNO_3	15 g.	$Mg\ SO_4.7H_2O$	3.6 g.		

[1] Contribution No. 236 from the Cereal Crops Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.

The nutrient agar was made as follows: 7 grams of agar were added to 1 litre boiling distilled water; 20 grams sucrose were added and stirred until dissolved; 5-ml. solution *A* and 2.5-ml. each of solutions *B* and *C* were added. When the solution cleared, a measured amount was poured into sterilized bottles ($1\frac{3}{4}$ inch x $2\frac{1}{2}$ inch) and the bottles plugged with rolled cotton. The bottled medium was sterilized in a pressure-cooker for 25 minutes at a pressure of 15 pounds and then stored in a refrigerator until used.

Other compounds were added to the medium in an attempt to produce a better substrate, *e.g.* coconut milk was added in one experiment, but since it was then not possible to sterilize the medium, contamination by fungi and bacteria prevented its use.

The procedure for excising the embryo and transferring it to the agar medium was as follows:

1. The transfer room was sprayed with a mixture of "Lysol" and water (1:3) 15 to 20 minutes prior to use. Tools were sterilized in alcohol or by passing through a flame. Just before use, the table surface, operator's hands, tools, etc., were washed with disinfectant ("S.T. 37", 50% solution).
2. Harvested seeds were disinfected by immersing for 1 minute in a commercial solution of chlorine (9 parts water: 1 part "Javex") and then transferred to another Petri dish containing distilled water.
3. Actual excision was done under a dissecting microscope, 18x. This operation requires considerable skill because any injury to the embryo reduces the chances of success.
4. The excised embryo was then placed on the agar surface, the opening of the bottle flamed, and the cotton plug replaced. It is not necessary to scratch or disturb the surface of the agar, nor is it advisable to pass the excised embryo through disinfectants or sterile water before placing it on the agar.
5. Bottles with embryos were placed in an incubator at 26°C. and examined periodically. After the embryos had germinated, the bottles were placed under fluorescent light. When roots and leaves were well formed the young plants were transferred to soil in pots.

Two methods were used for crossing: the approach method and hand pollination. However, in the approach method, the lemma and palea are clipped off and the seed often develops an abnormal shape. This caused some difficulty in excising the embryo and therefore the approach method was abandoned in favour of hand pollination.

RESULTS

The first *Hordeum* interspecific crosses were made in the greenhouse during the winter of 1951–52. Seeds were set in a number of crosses but they degenerated after an initial development. The experience obtained with these crosses plus information obtained from other workers (3,6,7) indicated that embryo culture would increase the chances of establishing hybrid populations from interspecific crosses. This technique has been used for most of the crosses in this program.

To use embryo culture technique to the best advantage the embryos must be left on the plants for optimum development and then removed before the seeds start to degenerate. After pollination, the rate of development of seeds varies with different hybrids and in different environments.

It is important that the seeds be removed from the plant at the optimum stage of development. Characteristics of colour and shape, once they are known, are useful in determining the optimum time for removal of the seeds. Practical experience is required to determine the correct time for seed removal. As a general rule, the seeds should be removed 8–12 days after pollination.

A number of parthenocarpic seeds developed in *Hordeum* crosses after the stimulation of pollination. Whether or not a swollen ovary on a spike contained an embryo was difficult to ascertain unless it was examined under the microscope. In some species there was more parthenocarpic development than in others, but differences also existed in different years.

The details of the hybridization program are given in Table 1. Some explanation is required to clarify points concerning the entries. The anomalous cross of *H. murinum* (*leporinum*) x *H. vulgare* (5), made in 1951–52, produced two types of segregates: murinum-like segregates and vulgare-like segregates. Both types are referred to as I.H. 5. I.H. 13 to I.H. 58 are hybrids produced by backcrossing specific lines and varieties of *H. vulgare* to vulgare-like segregates of I.H. 5. A cross between two *murinum* collections, *H. murinum*, B381 x *H. murinum*, B52, was made in the winter of 1952–53. The seeds developed normally and the embryos were not excised. The plants derived from this cross were fertile and have been designated I.H. 3. During 1957–58, *H. murinum*, B381 was crossed with *H. leporinum*, B501 and plants were produced without using embryo culture. This hybrid has been designated I.H. 92. Three collections of *H. bulbosum* are listed for the crosses made in 1952–53. At that time, two of these collections were maintained under other species names, i.e. B367 as *H. ischnatherum* and B55 as *H. ithaburense*. All entries listed under *H. bulbosum* have 4x = 28 chromosomes. In addition to the hybrids produced by direct crossing, one arose spontaneously in *H. pusillum* and is designated I.H. 93.

During the course of this work, attempts were made to produce amphiploids from the sterile hybrids by using colchicine and acenaphthene as well as heat shocks and x-ray treatment. These have been unsuccessful, although some doubled sectors were produced and some seeds were formed, which later degenerated. Sufficiently good pollen was produced in I.H. 4 and I.H. 6 to be used for pollination.

A summary of the crosses is given in Table 2. These data indicate where certain barriers to crossability exist as well as those cases where interspecific hybrids have been produced most successfully. Of the 39 different combinations attempted, 14 hybrids were obtained. Four hybrids were from direct crosses and three were from indirect crosses with *H. jubatum*. *H. depressum* and the related hexaploid species were involved as parents in four hybrids. *H. vulgare* produced hybrids with seven other species. *H. murinum* formed no viable hybrids with any other species. This is all the more surprising because more attempts were made to cross *H. murinum* than any other species. Crosses with *H. marinum*, as well as with the related *H. maritimum* and *H. hystrix* group, did not produce

TABLE 1.—INTERSPECIFIC AND INTERGENERIC CROSSES ATTEMPTED AND HYBRIDS OBTAINED THROUGH EMBRYO CULTURE IN THE PERIOD 1952–58

Cross	Seeds harvested	Embryos transplanted	Embryos germinated	Plants survived	Present status
1952–53					
H. vulgare, 5025 × *H. bulbosum*, B367		39	1	1	I.H.7
" " , Velvon × " " "		53	2	1	I.H.6
" " , Montcalm × " " "		38	5	2	I.H.2
" " , 5231 × " " "		19	5	5	I.H.11
" " , 5303 × " " "		43	3	0	
" " , Montclam × " " , B55		49	3	2	I.H.12
" " , 5303 × " " "		39	2	1	I.H.4
" " , 4811 × " " , B144		32	1	1	I.H.8
" " , 5231 × " " "		28	6	3	I.H.10
" " , 5303 × " " "		48	8	0	
" " , 5089 × " " "		58	3	2	I.H.1
H. spontaneum nigrum, B164 × *H. bulbosum*, B144		36	6	1	I.H.9
1953–54					
H. murinum, B381 × *H. vulgare*, 5089	16	9	7	0	
" " " × " " , 5090	38	27	16	0	
" " " × " " , 5343	16	13	12	0	
" " " × " " , C54–18	2	2	1	0	
" " " × " " , 2-rowed B208	12	12	6	0	
" " " × " " , Fort	20	20	5	0	
" " " × " " , I.H.5	55	40	24	0	
" " " × *H. bulbosum*, B55	68	19	3	0	
" " , I.H.3 × " " "	46	14	5	0	
" " " × " " , B367	16	2	2	0	
" " " × " " , B144	7	0	0	0	
" " " × *H. vulgare*, I.H.5	8	5	5	0	
" " " × " " , 5343	26	13	1	0	
" " I.H.5 × " " , Fort	31	31	13	0	
" " " × " " , 5090	35	30	30	0	
" " " × " " , I.H.5	3	3	3	0	
" " " × " " , 5343	49	44	22	0	
" " " × " " , 5425	25	15	11	0	
" " " × " " , 5427	16	15	9	0	
H. stenostachys, B56 × *H. vulgare*, C54–18	17	0	0	0	
" " " × " " , 5343	29	6	0	0	
" " " × *H. bulbosum*, B55	19	0	0	0	
" " " × " " , B144	41	3	0	0	
" " " × *H. pusillum*, B139	5	2	0	0	
H. pusillum, B139 × *H. vulgare*, C54–18	8	3	0	0	
" " " × " " , Fort	3	2	1	0	
" " " × *H. bulbosum*, B55	5	2	0	0	
" " " × *H. stenostachys*, B56	18	6	0	0	
H. marinum, B51 × *H. stenostachys*, B56	2	2	1	0	
" " " × *H. bulbosum*, B144	6	1	0	0	
" " " × " " , B367	33	1	1	0	
" " " × *H. vulgare*, 5343	34	34	16	0	
" " " × " " , I.H.5	51	28	16	0	
H. maritimum, B205 × *H. vulgare*, C54–18	11	3	0	0	
H. vulgare, 5424 × I.H.4	6	6	6	0	
" " " × I.H.6	27	19	13	0	
" " , 5425 × I.H.4	10	10	2	0	
1954-55					
H. murinum, B381 × *H. vulgare*, 5233	34	21	0	0	
" " " × " " , 5025	36	28	6	0	
" " " × " " , I.H.56	22	22	18	0	
" " " × " " , I.H.55	208	204	87	0	
" " , I.H.5 × *H. vulgare*, 5233	31	6	0	0	
" " " × " " , 5025	3	3	3	0	
" " " × " " , I.H.55	16	11	9	0	
H. stenostachys, B56 × *H. vulgare*, 5233	35	0	0	0	
H. marinum, B51 × *H. vulgare*, 5025	21	0	0	0	

TABLE 1.—INTERSPECIFIC AND INTERGENERIC CROSSES ATTEMPTED AND HYBRIDS OBTAINED THROUGH EMBRYO CULTURE IN THE PERIOD 1952–58—*Continued*

Cross		Seeds harvested	Embryos transplanted	Embryos germinated	Plants survived	Present status
H. jubatum, B204	× *H. vulgare*, 5233	19	5	0	0	
" " "	× " " , I.H.56	23	23	23	4	I.H.78
" " "	× " " , I.H.55	42	39	33	33	I.H.77
" " "	× " " , 4808	97	74	72	69	I.H.79
" " "	× *H. brachyantherum*, B214	7	7	7	6	I.H.74
" " "	× *Hordelymus europaeus*, B198	5	4	4	4	I.H.75
H. brachyantherum, B214	× *H. vulgare*, 5025	36	31	29	2	I.H.81
H. vulgare, 5343	× *H. bulbosum*, B144	29	6	2	0	
" " , I.H.56	× *Secale cereale*	29	3	1	1	I.H.82
" " , I.H.55	× *H. bulbosum*, B144	119	29	3	2	I.H.76
H. depressum, B256	× *H. vulgare*, I.H.55	15	11	9	2	I.H.80
" " "	× " " , 4808	11	9	0	0	
H. pusillum, B139	× *H. vulgare*, I.H.55	13	7	0	0	
	1955–56					
H. murinum, B381	× *H. vulgare*, 5233	65	57	21	0	
" " "	× " " , Kenate	83	69	42	0	
" " "	× " " , B557	8	6	5	0	
" " "	× " " , 4x = 28	28	0	0	0	
" " "	× *Secale cereale*	12	7	0	0	
" " "	× I.H.1	23	21	14	0	
" " "	× *Elymus racemosus*, B358	28	14	0	0	
I.H.74 F_1	× *H. vulgare*, Montcalm	14	10	8	6	I.H.86
"	× " " , Kenate	32	7	3	0	
"	× " " , O.A.C.21	68	39	5	0	
"	× Hexaploid species, B562	9	7	5	2	I.H.90
I.H.74 F_2	× *H. vulgare*, Kenate	68	57	45	41	I.H.84
"	× " " , Fort	33	29	17	16	I.H.85
"	× " " , 5233	57	33	9	8	I.H.83
"	× *H. californicum*, B257	8	8	5	5	I.H.87
H. glaucum, B1040	× *H. vulgare*, Kenate	31	0	0	0	
H. hystrix, B254	× *H. vulgare*, Kenate	38	0	0	0	
H. depressum, B256	× *Secale cereale*	5	4	4	2	I.H.89
H. californicum, B257	× *Secale cereale*	8	0	0	0	
Hexaploid species, B562	× *H. vulgare*, O.A.C.21	11	9	8	6	I.H.88
" "	× " " , Fort	15	8	0	0	
H. marinum, B51	× *H. vulgare*, 5233	26	19	0	0	
" " "	× " " , Kenate	11	9	0	0	
" " "	× Hexaploid species, B562	9	9	0	0	
	1956–57					
I.H.90	× *H. vulgare*, Dorsett	1	1	0	0	
H. maritimum, B458	× *H. vulgare*, 5025	51	1	0	0	
H. murinum, B381	× *H. vulgare*, 5230	12	9	0	0	
" " "	× " " , Parkland	39	38	28	0	
" " "	× " " , Ricardo	39	38	8	0	
" " , B366	× " " , Montcalm	35	27	0	0	
" " "	× " " , Parkland	29	26	22	0	
	1957–58					
H. murinum, B381	× *H. vulgare*, Kearney	226	211	56	0	
" " "	× " " , Dicktoo	97	62	28	0	
" " "	× " " , I.H.5	230	194	83	0	
" " "	× *H. spontaneum nigrum*, B164	13	12	0	0	
" " , B649	× *H. vulgare*, Kearney	81	79	41	0	

TABLE 1.—INTERSPECIFIC AND INTERGENERIC CROSSES ATTEMPTED AND HYBRIDS OBTAINED THROUGH EMBRYO CULTURE IN THE PERIOD 1952–58—*Concluded*

Cross	Seeds harvested	Embryos transplanted	Embryos germinated	Plants survived	Present status
1957-58					
H. leporinum, B501 × *H. vulgare*, Kearney	12	9	5	0	
" " " × *H. stenostachys*, B56	6	0	0	0	
Hexaploid species, B562 × *H. vulgare*, Reno	9	4	0	0	
" " × " " , Kearney	19	3	0	0	
I.H.74 F_2 × *H. vulgare*, Dicktoo	29	21	8	1	I.H.91
" × *H. murinum*, B381	6	4	0	0	
H. vulgare, I.H.5 × *H. bulbosum*, B830	33	16	0	0	
" " , Kearney × " " " , B843	39	13	0	0	
" " , Dicktoo × " " " , "	27	8	0	0	
H. jubatum × unknown, B255 ?	13	3	1	1	I.H.94

TABLE 2.—SUMMARY OF INTERSPECIFIC AND INTERGENERIC CROSSES ATTEMPTED AND HYBRIDS OBTAINED THROUGH EMBRYO CULTURE, IN THE PERIOD 1952–58

Cross	Hybrids
H. brachyantherum × *H. vulgare*	*
H. californicum × *Secale cereale*	*
H. depressum × *H. vulgare*	*
" " × *Secale cereale*	*
Hexaploid species × *H. vulgare*	*
H. jubatum × *H. vulgare*	*
" " × *H. brachyantherum*	*
" " × *Hordelymus europaeus*	*
" " × unknown	*
I.H. 74 × *H. vulgare*	*
" × Hexaploid species	*
" × *H. californicum*	*
" × *H. murinum*	—
I.H. 90 × *H. vulgare*	—
H. marinum × *H. vulgare*	—
" " × *H. bulbosum*	—
" " × *H. stenostachys*	—
" " × Hexaploid species	—
H. maritimum × *H. vulgare*	—
H. hystrix × *H. vulgare*	—
H. murinum × *H. vulgare*	—
" " × *H. vulgare* (4x)	—
" " × *H. bulbosum*	—
" " × *H. stenostachys*	—
" " × *Secale cereale*	—
" " × *Elymus racemosus*	—
" " × I.H. 1	—
H. glaucum × *H. vulgare*	—
H. pusillum × *H. vulgare*	—
" " × *H. bulbosum*	—
" " × *H. stenostachys*	—
H. stenostachys × *H. vulgare*	—
" " × *H. bulbosum*	—
" " × *H. pusillum*	—
H. vulgare × *H. bulbosum*	*
" " × *Secale cereale*	*
" " × I.H.4	—
" " × I.H. 6	—
H. spontaneum × *H. bulbosum*	*

* Indicates hybrid plants produced

any hybrids, nor did crosses with *H. stenostachys* and *H. pusillum*. *H. bulbosum* produced hybrids readily with *H. vulgare* and *H. spontaneum*, a close relative of *H. vulgare*.

DISCUSSION

The data in Table 1 indicate that certain crosses did not produce viable embryos although seed setting was adequate. At least we were unsuccessful in culturing embryos from certain crosses. Some of this failure to obtain germination may be due to lack of suitable techniques for embryo excision as well as to incorrect timing regarding the removal of seeds from the plant. For example, in the cross *H. stenostachys* x *H. vulgare*, 5233, 35 seeds developed but the embryos had degenerated before being removed from the plant. Another reason for the difference between the number of harvested seeds and transplanted embryos is that many parthenocarpic bodies were produced. When dissected, such bodies contained fluid or had only rudimentary embryo development. The incompatibilities, as shown in Table 1, should be assessed in the light of this information.

Another difficulty encountered in rating the compatibility of any two species is that the floral parts of many species are very small and it is difficult to make a large number of crosses. Therefore, the crosses and reciprocal crosses have not been rated in terms of incompatibility with any one parent as Davies (4) has done. During the course of this experiment many different varieties and lines of *H. vulgare* and different strains of the species, *H. murinum* and *H. bulbosum*, have been used in a number of combinations. In some instances hybrids were produced but it is not certain whether or not some combinations are more compatible than others.

Certain abnormalities were observed when the embryos germinated. Some grew only roots and others only coleoptiles. In a large number of embryos both roots and coleoptiles developed and the plants appeared healthy but, after some growth, development ceased and the plants died. The length of time that elapsed before growth ceased varied from a few days to 6 weeks. In an effort to overcome this difficulty a number of techniques were tried, such as: the plants being retained on nutrient agar; nutrient solutions were added to the soil; plants were cold treated or given colchicine injections; etc. However, in no case was the life of the plant prolonged for more than a week. Crosses between *H. murinum* and *H. vulgare* have given this same result for 6 years. Actual seed set in this cross was often as high as 60 per cent. At the time of excision, the embryos looked firm and healthy, a condition which is normally reflected in good germination. These results are similar to those obtained by Davies (4). Using embryo culture, he was able to produce plants from the crosses of *H. californicum* x *H. vulgare*, *H. californicum* x *H. bulbosum*, and *H. vulgare* x *H. bulbosum*. Some plants did not produce roots, others grew slowly for a few weeks, and some appeared normal, but all died before maturity.

A few of the plants produced in this program were examined cytologically and chromosome counts indicated that they were hybrids. It was difficult to find dividing cells and in many of the root tips examined there appeared to be a complete breakdown of mitotic divisions.

Since the plants do not survive past the seedling stage it can be assumed that either: 1, The two genomes are incompatible in a common cytoplasm; or 2, A lethal factor is operative at a certain stage in the development of the plant. If the latter assumption is correct, then the failure of the hybrid plants to reach maturity does not mean that the species are not related. It does indicate, however, that a criterion other than hybridity must be used to assess species relationships.

Fourteen different hybrids, most of them completely sterile, were grown to adult plants. How do these hybrids compare with those obtained by other workers? The excellent review by Smith (10) shows that viable hybrids have been reported for: *H. jubatum* x *H. vulgare*; *H. vulgare* x *H. bulbosum*; *H. nodosum* x *H. vulgare*; *H. compressum* x *H. stenostachys*; and *H. jubatum* x *S. cereale*. In addition, hybrids have been obtained between *Elymus* and *Hordeum* species including *H. nodosum* x *Elymus glaucus*. Hybrids between *H. vulgare* and *H. bulbosum* were reported by Konzak *et al.* (6). A number of reports indicate that *H. jubatum* and *H. vulgare* have been crossed successfully. Bowden (1) reported that *Elymus* species will cross with *H. jubatum*, *H. brachyantherum* and *H. distichon*. Boyle and Holmgren (2) studied the cross *H. jubatum* x *Agropyron trachycaulum*.

It is apparent that a number of successful crosses have been made with the native weed, *H. jubatum*. It combines readily with other species of *Hordeum* and has some affinities with *Elymus*, *Hordelymus*, *Agropyron*, and *Secale*. *H. brachyantherum* has also been crossed successfully with other genera and it may prove to be a useful species to bridge the gap between *Hordeum* species. The hybrid *H. jubatum* x *H. brachyantherum*, produced in this program, is partially fertile indicating the close relationship of these two species. *H. depressum* and the related hexaploid species, which may also be related to *H. brachyantherum*, have been fruitful in crosses. *H. murinum* was used extensively in crosses. With *H. vulgare* it certainly gave good seed set and the excised embryos grew well for a time but because hybrid plants could not be maintained it is concluded that it is not closely compatible with other species.

With the exception of *H. vulgare* x *S. cereale*, all the interspecific and intergeneric crosses studied in this program had at least one parent with 28 chromosomes. Future work will include doubling of the diploid species and using them in crosses. More crosses will also be made between wild species of *Hordeum* rather than between *H. vulgare* and other wild species.

ACKNOWLEDGEMENT

D. G. Hamilton initiated this program of research in the genus *Hordeum* and was responsible for the progress until 1955. It is a pleasure to acknowledge his assistance, his helpful criticism, and the active interest he has maintained in this project.

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GERMINATION OF ALFALFA VARIETIES IN SOLUTIONS OF VARYING OSMOTIC PRESSURE AND RELATIONSHIP TO WINTER HARDINESS[1]

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ABSTRACT

Two laboratory experiments were conducted to evaluate the reliability of amount of germination in solutions of varying osmotic pressure, as a means of separating alfalfa varieties into winter-hardiness classes. In one test 23 varieties or strains were studied, and in the other 36. It was found that significant differences exist between certain alfalfa varieties in their ability to germinate in sucrose or sodium chloride solutions of 3, 6, and 9 atmospheres. There is a general tendency for non-hardy varieties to germinate more rapidly and more completely than hardy ones but there are many exceptions to this trend. Germination in solutions of 6 atmospheres osmotic pressure at 5 days gave the best separation of varieties on the basis of their ability to germinate. Germination was generally better in solutions of sucrose at 6 atmospheres osmotic pressure than in solutions of sodium chloride of the same osmotic pressure but several varieties germinated equally well in either solution. The results indicate that germinating alfalfa in sugar or salt solutions is not a reliable method for differentiating alfalfa varieties into winter hardiness classes.

INTRODUCTION

In 1957, Rodger *et al.* (6) reported a method for evaluating winter hardiness in alfalfa, based on differences in rapidity and amount of germination in solutions of sugar (sucrose) and salt (sodium chloride) of definite osmotic pressure. They found that as the osmotic pressure of solutions increased, the speed and amount of germination of seed of all varieties decreased. This decrease, however, was much more marked in seed of hardy varieties than of non-hardy ones. If the method is reliable within fairly narrow limits, it would be very useful in classifying newly developed strains and varieties of alfalfa for winter hardiness and consequently would minimize the necessity for field tests of some of them in cold climatic regions where winter hardiness in a variety is of major importance.

In order to provide further information on the reliability of the method two laboratory tests were undertaken at Swift Current, Saskatchewan. One was conducted in 1957 with 23 varieties or strains, using sugar (sucrose) solutions at three levels of osmotic pressure, and the other in 1958 with 36 varieties using sugar (sucrose) and salt (sodium chloride) solutions at one level of osmotic pressure. The techniques used in the experiments followed those of Rodger *et al.* (6) closely with minor variations.

MATERIALS AND METHODS

Varieties Used and Their Field Winter Hardiness Reaction

The alfalfa varieties and strains used in the two experiments reported in this paper have been under observation for relative winter hardiness

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TABLE 1.—WINTER HARDINESS OF ALFALFA VARIETIES FROM FIELD OBSERVATIONS AT SWIFT CURRENT AND SASKATOON, 1938 TO 1958 INCLUSIVE

Variety or strain	Number of tests in which variety occurred	Average winter-killing %*	Winter hardiness rating** Mean of tests	Author's winter hardiness rating***
Siberian	9	0	3.1	1
Syn. Sc 3513	5	2	.2	1
Rambler	5	8	.5	2
Paramount	2	1	4.0	3
Ladak	28	25	4.0	4
Grimm	23	32	4.2	4
Rhizoma	16	37	4.6	5
Vernal	7	36	4.3	5
Hardistan	6	23	5.0	5
Hardigan	1	46	6.0	5
Viking	5	17	5.0	5
Ferax	6	49	5.0	5
Ontario Variegated	2	26	5.0	5
Canauto	3	9	4.3	5
Sevelra	8	24	5.0	6
Ranger	11	40	5.0	6
Buffalo	6	50	7.7	7
Nomad	7	47	7.6	7
Atlantic	3	38	7.0	7
Du Puits	6	64	8.5	8
Flemish Socheville	1	100	13.0	8
Talent	7	75	10.0	8
Lahontan	2	88	12.5	9
Arizona Chilean	3	67	10.3	9
Pilca Butta	2	62	10.0	9
South African High'd	1	85	12.0	9
Australian Common	2	96	13.5	10
New Zealand Common	2	95	14.0	10
Indian Common	2	100	14.0	10

*Disease was not a contributory factor to winter injury in any of the tests being considered.
**Basis of calculation for each test was: winter-killing of Ladak (a common variety in all tests) = 4; other varieties were given a value of 1, up or down from 4, for each 10% difference in winter-killing.
***Author's rating 1 to 10 is based on data presented in the table, adjusted somewhat in the light of published data (1, 2, 3, 4, 5) and general observations; 1 = most hardy and 10 = least hardy.

in the Prairie Provinces of Canada for several decades in the case of older varieties, and for at least 5 years in the case of those developed more recently. Winter killing of alfalfa in the drier regions of the Prairie Provinces is generally the result of cold effect plus desiccation, and seldom is it complicated by disease effects, icing effects, or heaving. Field observations on winter injury in this region, therefore, are perhaps as reliable as any obtainable anywhere for assessing winter-hardiness qualities.

In Table 1 data are presented from 21 field tests in which winter injury occurred at Swift Current, located in southwestern Saskatchewan and from 7 tests at Saskatoon, located in central Saskatchewan. At both locations the mean annual precipitation is about 14 inches but the climate at Swift Current is considered to be more arid because of a much greater evaporation rate. The writer used these data along with published information on winter hardiness (1, 2, 3, 4, 5) to arrive at the rating for winter hardiness of the varieties presented in the last column of Table 1.

TABLE 2.—GERMINATION OF ALFALFA VARIETIES IN SUGAR SOLUTION OF THREE ATMOSPHERES OSMOTIC PRESSURE AS A PER CENT OF GERMINATION IN DISTILLED WATER—1957 LABORATORY TEST

Variety or strain	Variety code letter	Hardiness rating	Germination as a per cent of germination in distilled water—conv. to $\sin^2\theta$					
			3 days	4 days	5 days	6 days	7 days	Final
Siberian Sc 1531	A	1	27	32	39	45	48	53
Siberian Sc 1522	B	1	40	44	46	54	56	55
Syn. Sc 3513	C	1	28	38	40	46	49	58
Rambler	D	2	50	52	60	66	69	69
Paramount Sc 1535	E	3	50	52	60	70	73	73
Ladak	F	4	56	60	71	74	76	77
Grimm	G	4	46	52	42	68	72	72
Rhizoma	H	5	31	38	53	60	66	69
Vernal	I	5	56	59	70	75	77	78
Hardistan	J	5	72	75	77	78	79	79
Hardigan	K	5	47	51	55	58	58	58
Viking	L	5	37	42	53	60	63	68
Ferax	M	5	50	54	60	62	66	69
Ontario Variegated	N	5	49	53	59	60	63	63
Canauto	O	5	54	53	58	64	67	69
Sevelra	P	6	60	61	70	71	78	77
Ranger	Q	6	66	68	76	79	80	81
Buffalo	R	7	57	57	64	72	73	73
Nomad	S	7	48	53	59	70	71	74
Du Puits	T	8	57	58	66	70	74	74
Flemish Socheville	U	8	54	58	59	69	70	72
Lahontan	V	9	62	66	77	80	83	84
Australian Common	W	10	63	68	75	81	82	83
S.E. Mean			5.9	6.0	6.2	7.0	7.0	6.9
C.V.			23.5	22.3	20.2	21.0	20.2	19.4
Varieties differing significantly at P= .05 calculated according to Newman-Keule Multiple Range Test			J>A,B,C,H & L Q,W,V>A,C & H P>A,C	J>A,C,H & L Q,W,V>A	J,Q,V & W> A,C & G	N.S.	N.S.	N.S.

TABLE 3.—GERMINATION OF ALFALFA VARIETIES IN SUGAR SOLUTION OF SIX ATMOSPHERES OSMOTIC PRESSURE AS A PER CENT OF GERMINATION IN DISTILLED WATER—1957 LABORATORY TEST

Variety or strain	Variety code letter	Hardiness rating	Germination as a per cent of germination in distilled water—conv. to $\sin^2\theta$					
			3 days	4 days	5 days	6 days	7 days	Final
Siberian Sc 1531	A	1	8	12	20	22	28	29
Siberian Sc 1522	B	1	23	24	34	39	44	44
Syn. Sc 3513	C	1	12	15	18	26	36	39
Rambler	D	2	24	27	34	43	46	46
Paramount Sc 1535	E	3	16	16	31	34	35	36
Ladak	F	4	29	32	44	54	59	58
Grimm	G	4	21	24	31	37	41	42
Rhizoma	H	5	19	20	28	36	40	41
Vernal	I	5	18	28	32	37	44	47
Hardistan	J	5	41	47	70	72	78	78
Hardigan	K	5	21	24	29	32	36	36
Viking	L	5	18	23	34	43	44	47
Ferax	M	5	12	17	30	43	46	46
Ontario Variegated	N	5	20	26	32	37	37	38
Canauto	O	5	19	22	32	39	43	43
Sevelra #3	P	6	34	34	43	50	54	55
Ranger	Q	6	37	40	48	54	59	57
Buffalo	R	7	33	38	49	58	62	64
Nomad	S	7	16	25	34	41	45	47
Du Puits	T	8	26	32	42	48	51	51
Flemish Socheville	U	8	25	31	46	50	52	56
Lahontan	V	9	38	44	55	53	53	53
Australian Common	W	10	31	39	43	49	52	54
S.E. Mean			4.9	5.4	4.5	5.2	5.8	6.0
C.V.			41.8	39.0	24.0	24.0	24.5	24.7
Varieties differing significantly at P = .05 calculated according to Newman-Keule Multiple Range Test			J & V>A,C & M P & R>A	J>A,C,E & M V>C & E	J>all others S& V>A to 0 except F & J F,P,Q,R,U & W>A & C T>C	J>A to 0 except F F,Q,R & V >A & C P,T,U & W >A	J>A to 0 except F F,Q & R> A	J>A to 0 except F R>A

1957 Experiment

Twenty-three alfalfa varieties were studied for germinability in sugar solutions of 3, 6, and 9 atmospheres osmotic pressure in a 4-replicate test using a split-plot arrangement. The varieties constituted the main plots and the sugar solution treatments the sub-plots. A check using distilled water was included with each set of the three sugar solutions and was used to allow for expression of germination in the sugar solutions as a percentage of germination in distilled water. This adjusted for initial differences in germination of varieties which did vary from a low of 41 per cent for a Siberian strain to a high of 94 per cent for Lahontan. Seed was not scarified. Four lots of 100 seeds were placed on two Whatman #4 filter papers in 9-cm. petri dishes for each treatment. The filter papers were saturated by adding 4 cc. of the required solution to each petri dish at the start of the experiment and 2 cc. 4 days later. The experiment was conducted on benches in a laboratory in which the temperature varied between 70° and 80° F. Germination counts were made at 24-hour intervals starting on the second day. The plot data were converted to $\text{Sin}^2\,\theta$ prior to statistical analysis because of variability within wide limits. The data for each solution concentration were analysed separately.

1958 Experiment

In this experiment 13 varieties or strains were included in addition to those used in the 1957 experiment. This was done to provide a still broader picture of differences between varieties in their ability to germinate in solutions of definite osmotic pressure. Since the 6-atmospheres osmotic pressure solution gave the best differential between varieties in the 1957 experiment, it was the only one used in the 1958 experiment. However, all varieties and strains were germinated in solutions of salt (sodium chloride) as well as sugar (sucrose). The design of the test was a 4-replicate split-plot type in which the varieties constituted the main plots, and the two solutions of sugar and salt the sub-plot treatments. As in the 1957 experiment, 100 seeds were germinated in distilled water with each set of sugar and salt solution and were used to make calculations of germination in the solutions as a per cent of germination in distilled water. The amount of solution added to each petri dish and the filter paper used were the same as in the first test, as was the temperature in the laboratory and the frequency of counting. In order to minimize effects of mould the seed to be soaked with sugar solution was lightly treated with arasan. Although some of the seed was nearly 20 years old germination was quite good; the lowest germination for old seed (1939) was for Ferax 59 per cent (Table 5, Column 3). None of the seed was scarified and it was observed that germination was quite low for several strains which contained a considerable amount of *M. falcata* germ plasm, because of hard seeds, i.e., Siberian, Rambler, and Paramount.

RESULTS AND DISCUSSION

1957 Experiment

The germination data were subjected to a variance analysis separately for each sugar concentration treatment and each day starting with the third day count for the 3- and 6-atmospheres osmotic pressure treatment,

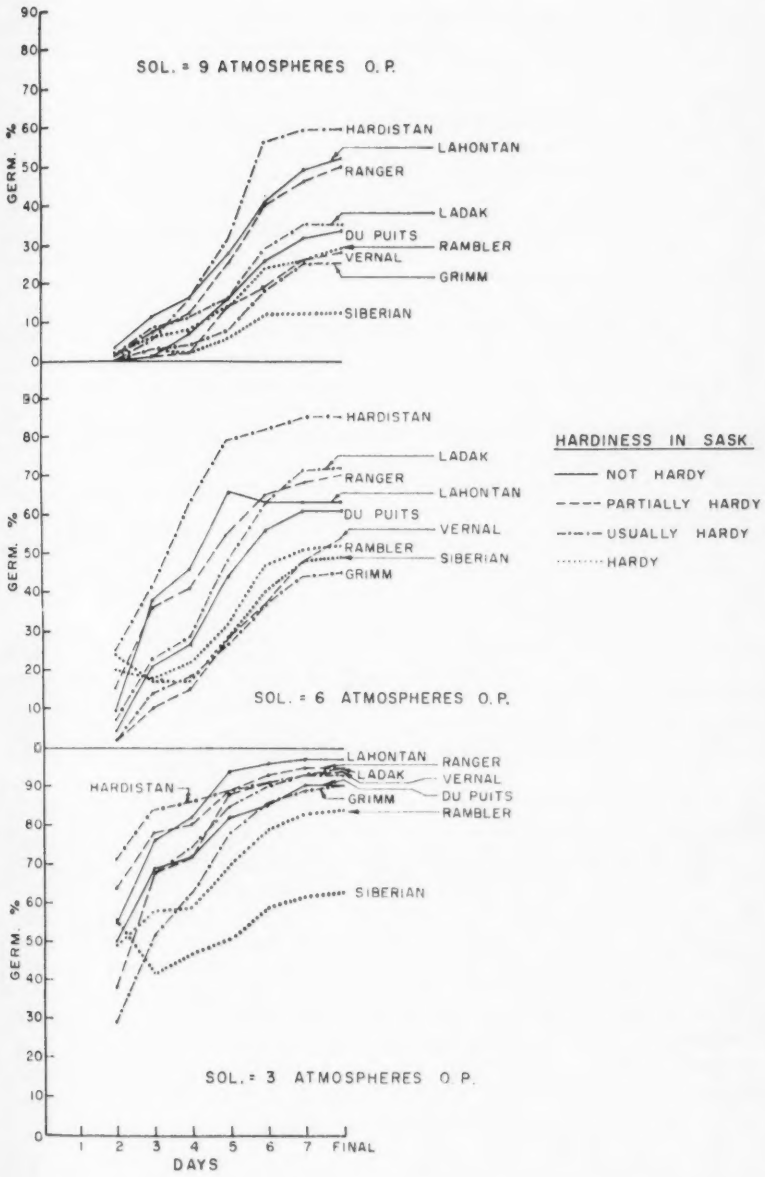

FIGURE 1. Germination of 9 alfalfa varieties in sugar solution of varying osmotic pressure as a percentage of germination in distilled water.

TABLE 4.—GERMINATION OF ALFALFA VARIETIES IN SUGAR AND SALT SOLUTIONS OF SIX ATMOSPHERES AS A PER CENT OF GERMINATION IN DISTILLED WATER—1958 LABORATORY TEST

Variety or strain	Seed		Hardiness rating 1–10	Germination as a per cent of germination in distilled water—conv. $\sin^2\theta$					
				Five days			Final		
	Age	1958 Germ. %		Sugar	Salt	Mean	Sugar	Salt	Mean
Siberian Sc 1531	1953	57	1	30	24	27	45	37	41
Siberian Sc 1522	1952	35	1	20	22	21	35	33	34
Syn. Sc 3513	1954	39	1	24	7	16	44	23	34
Rambler F 2542	1954	58	2	48	36	42	60	46	53
Rambler St. Valley	1956	60	2	30	32	31	56	48	52
Rambler St. Valley	1957	52	2	39	43	41	57	60	59
Rambler Cal.	1956	68	2	38	26	32	66	46	56
Rambler Cal.	1957	69	2	44	36	40	65	50	58
Rambler Humboldt	1957	38	2	27	37	32	46	55	50
Paramount	1954	49	3	27	16	22	43	33	38
Ladak	1951	86	4	50	43	47	66	60	63
Grimm (Com.)	1951	82	4	54	30	42	67	46	56
Grimm (Saskatoon)	1957	92	4	55	33	44	81	49	65
Rhizoma	1951	71	5	39	18	28	59	44	51
Vernal	1956	87	5	56	40	48	69	52	60
Hardistan	1939	63	5	60	42	51	71	56	64
Hardigan	1939	79	5	59	32	45	68	42	55
Viking	1939	80	5	47	36	42	64	46	55
Ferax	1939	59	5	43	14	29	59	26	43
Ont. Variegated	1939	72	5	51	28	40	73	43	58
Canauto	1939	78	5	50	26	38	69	38	54
Sevelra	1951	82	6	54	34	44	67	52	60
Ranger	1951	79	6	56	52	54	64	58	61
Buffalo	1951	87	7	67	45	56	75	58	67
Nomad	1953	76	7	45	31	38	60	42	51
Atlantic	1951	75	7	72	51	62	82	61	71
Du Puits	1951	65	8	63	40	52	68	50	58
Flemish Socheville	1955	72	8	58	32	45	67	46	56
Talent	1951	78	8	72	59	65	74	73	74
Lahontan	1954	81	9	66	44	55	82	58	70
Arizona Chilean	1951	92	9	71	50	60	79	56	68
Pilca Butta	1951	60	9	64	28	46	76	48	62
South African High'd	1950	80	9	60	36	48	74	54	64
N.Z. Common	1950	70	10	52	34	43	72	48	60
N.Z. Marlborough	1951	68	10	54	33	44	84	48	66
Indian Common	1947	78	10	68	52	60	76	56	66
S.E. Mean				—	—	4.6	6.2	7.1	5.3
C.V.						28.7	19.3	30.6	26.2

and with the fifth day count for the 9-atmospheres osmotic pressure treatment. Earlier counts were not analysed because of great variability and numerous zero values. Occasional zero values which occurred in the data that were analysed were given a .1 value and then converted to $\text{Sin}^2\,\theta$.

Germination in solution of 3-atmospheres osmotic pressure (Table 2) gave best varietal separation when counted on the third day, followed by the 4- and 5-day counts, respectively. No significant differences between varieties in germination were obtained from the sixth, seventh, and final day counts. It will be noted that the coefficient of variability decreased only slightly for data from later day counts as compared to the 3- and 4-day counts.

Germination, in sugar solutions of 6-atmospheres osmotic pressure (Table 3), separated varieties on the basis of amount of germination much better than in solution of 3-atmospheres osmotic pressure (Table 2). The best separation occurred at 5 days followed closely by 6 days. The coefficient of variability was very great for data from 3- and 4-day counts and about the same for all others.

Germination in sugar solution of 9 atmospheres osmotic pressure was erratic and the experimental error was very great, the coefficient of variability being never less than 40 per cent at any counting time. It is too concentrated a solution to give separation of varieties into various germination classes.

The results indicate that germination in sugar solution of 6-atmospheres osmotic pressure, which appears to be the best concentration to give separation, is not a reliable method to classify varieties into hardiness classes. As a most glaring example, the variety Hardistan (J), of the medium hardy class, followed a germination trend that would classify it as a non-hardy type and according to the 5-day count its germination was significantly higher than that of any other variety. Other exceptions are readily apparent in the germination relationships since the varieties are listed in approximate order of hardiness.

In Figure 1 the inconsistencies are clearly illustrated. There are reversals of curve trends between varieties when considering germination at various sugar concentrations. In addition, varieties of a similar hardiness classification have vastly different germination curves. In some cases hardy varieties tend to follow non-hardy curve trends and in other cases non-hardy varieties follow curve trends of hardy varieties. It should be remembered by the reader that, according to the results obtained by Rodger *et al.* (6), the flatter the curve the hardier the variety.

The results obtained in this experiment partly verify results obtained by Rodger *et al.* (6) and partly they are completely at variance with them. It all depends which varieties are selected for comparison. There is a tendency, however, for non-hardy varieties to germinate better in sugar solutions than hardy ones, but there are many exceptions.

1958 Experiment

The data for the 5-day and final germination counts are presented in Table 4. Highly significant differences in germination between varieties were observed at both times. Germination was much better in sugar

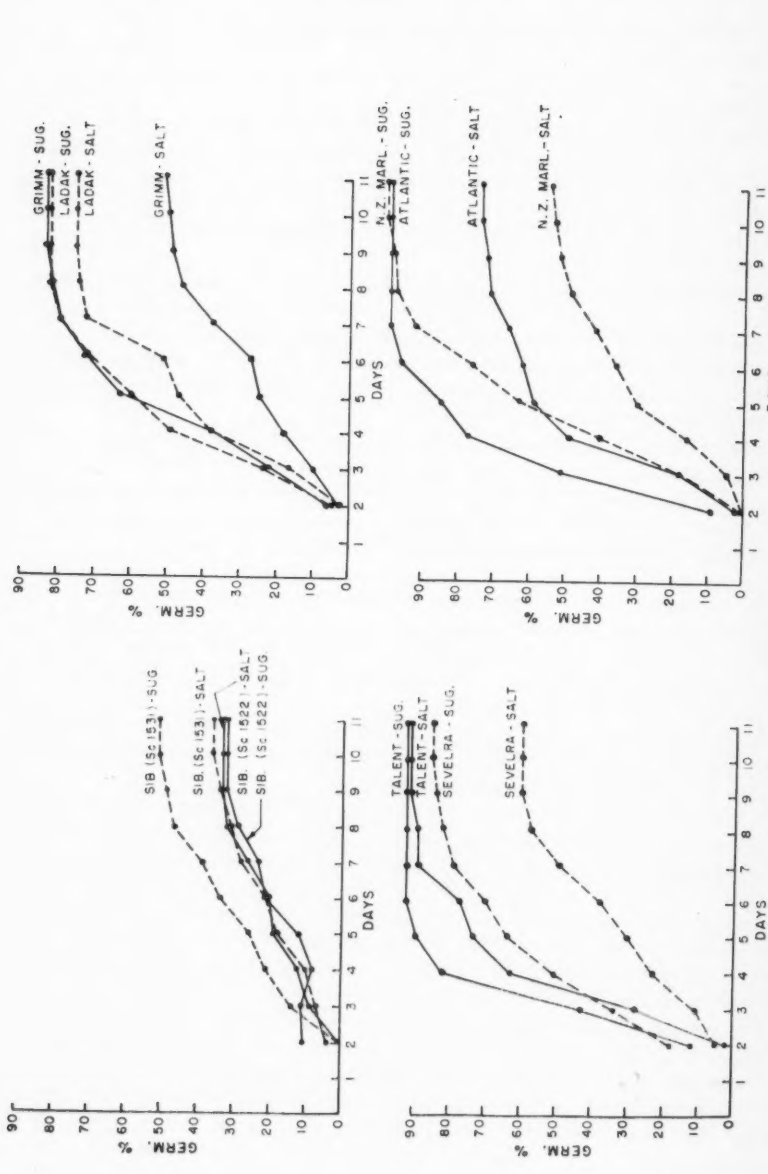

FIGURE 2. Germination of alfalfa varieties in solutions of sugar and sodium chloride, of 6-atmospheres osmotic pressure, as a percentage of germination in distilled water, depicting interaction of varieties x sugar or salt solution.

solution than in the salt solution but there was a significant variety x sugar or salt interaction. The interaction trend is illustrated in Figure 2 in which the germination curves, for extreme varietal reaction, within similar hardiness groups have been plotted on a graph. It will be noted, too, from Table 4 that the coefficient of variability is greater for the salt solution portion of the test than for the sugar solution portion. The correlation coefficient between the germination counts in sugar solution and salt solution was +.87, significant at the 1 per cent level, which indicates quite good agreement between the two media.

Examination of the mean germination counts at 5 days and final reveals that there is a general tendency for non-hardy varieties to germinate better in solutions of 6-atmospheres osmotic pressure than hardy ones. Actually, the correlation coefficient between the mean germination (Table 4) and the hardiness ratings is +.71 significant at the 1 per cent level. However, it is also obvious that there were wide variations in germination potential in solutions of 6-atmospheres osmotic pressure within similar hardiness groups, and then again in many instances there were no differences between varieties differing widely in winter hardiness. For example, at 5 days, Rhizoma and Ferax germinated much lower than Hardistan and Vernal and yet all four are considered to be equally hardy in Saskatchewan, and although there are no germination differences between Grimm, Ladak, and the two New Zealand strains the latter two always winterkill during the first winter in Saskatchewan, while Grimm and Ladak have been widely used in Alberta, Saskatchewan, and Manitoba during the last three decades and are still on the recommended list for use on non-irrigated land.

A correlation coefficient was calculated for final germination results in sugar solution of 6-atmospheres osmotic pressure as recorded for the 23 varieties common to both the 1957 and 1958 experiments in the two years and it was found to be +.67, significant at the 1 per cent point. This is a low r value and emphasizes the erratic nature of the results that can be expected.

CONCLUSIONS

1. Significant differences exist between alfalfa varieties, in their ability to germinate in sugar or salt solution of certain osmotic pressure, but differentiation into hardy and non-hardy groups by this method is unreliable.
2. There is a tendency for a greater number of non-hardy alfalfa varieties to germinate better in sugar and salt solutions of definite osmotic pressure than hardy ones. However, there are frequent exceptions to this tendency.
3. There is an indication that varieties containing a high proportion of *M. falcata* germ plasm (Siberian, Rambler, Syn. Sc 3513, and Paramount) as a group, tend to germinate poorly in sugar and salt solutions compared to varieties mostly of *M. sativa* origin (Ranger, Buffalo, Du Puits, Lahontan, New Zealand strains, and Indian Common).
4. Germination in sugar solution of 6 atmospheres osmotic pressure appears to give the best separation of varieties into classes as determined by germination in sugar and salt solutions of certain osmotic pressure.
5. The high standard errors in the tests and some lack of agreement between results from different tests suggest that environmental conditions should be precisely controlled in tests of this kind.

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VARIETAL AND ENVIRONMENTAL EFFECTS ON RAPESEED

I. ISOTHIOCYANATE AND THIOOXAZOLIDONE CONTENT[1]

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ABSTRACT

The isothiocyanate and thiooxazolidone content was determined on seven rapeseed varieties grown at seven locations across Western Canada. The isothiocyanate content varied significantly for varieties but not for stations whereas the thiooxazolidone content differed significantly for both varieties and stations. The Argentine-type could easily be distinguished from the Polish-type as the thiooxazolidone content was approximately three times greater in the former. No such varietal distinction was found for the isothiocyanate content.

INTRODUCTION

Rapeseed production for its oil content is becoming more and more important in Western Canada. Some of the reasons for the increased interest are: (*a*) the success with which it can be grown in Western Canada; (*b*) its ability to compete economically with wheat under the present economic conditions, and (*c*) rapeseed oil has been accepted for use in human foodstuffs. The last reason could mean that more rapeseed will be grown and processed in Canada and hence large stocks of the meal may be available for use as supplements in animal and poultry feeds.

Some experimental work points to the existence of toxic factors in rapeseed which affect the growth of many species of animals. Blakely and Anderson (4) reported that rapeseed oil meal caused an enlargement of the thyroid gland in turkey poults. Since then a number of reports (3, 8, and 11) indicate that rapeseed meal has a deleterious effect on the growth of various species. An excellent review on this subject has been published by Bell (2).

One factor known to be present in rapeseed has definite goitrogenic activity. Astwood and co-workers (1) reported that *l*-5-vinyl-2-thiooxazolidone derived from a glycoside found in brassica seeds causes an enlargment of the thyroid and impairs proper functioning of this gland. Raciszewski, Spencer, and Trevoy (9) found that this material was present in considerable quantities in rapeseed meal. Other sulphur compounds, the isothiocyanates also derived from glycosides, are present in rapeseed and one has been identified by Ettlinger and Hodgkins (6) and Kjaer and his group (7) as allyl-carbinyl isothiocyanate. The toxic effects of these compounds are not well defined and most of the evidence implicating them is of an indirect nature.

The interest in rapeseed has led plant-breeders to attempt to select for higher oil content and for lower thiooxazolidone and isothiocyanate content in varieties suitable for Western Canadian conditions. To aid this

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selection the isothiocyanate and thiooxazolidone contents of a number of varieties grown at Experimental Farms and Universities in Western Canada were determined and these data are presented in this paper.

MATERIAL AND METHODS

Samples of seven varieties were collected in the fall of 1956 from stations across Western Canada. The varieties Argentine, Golden, Regina II, and Swedish were of the Argentine-type (*Brassica napus* L.) and Polish, Arlo, and Gute were of the Polish-type (*Brassica campestris* L.). The stations selected were in the extreme south of Western Canada because of extensive frost damage at more northerly points. The rapeseed samples were bulked from rod rows grown at each station.

Approximately 10 grams of finely ground rapeseed were extracted overnight with Skellysolve "F"* on a "Goldfisch" fat extraction unit. The solvent was evaporated and the residual oil used for the determination of the fatty acid content (5). The meal was ground in a micro-Wiley mill, and stored in glass vials until used for analysis.

* Petroleum ether (b.p. range 35°–58°C.)

TABLE 1.—THE ISOTHIOCYANATE AND THIOOXAZOLIDONE CONTENTS OF DIFFERENT VARIETIES OF RAPESEED AT DIFFERENT STATIONS

Station variety		Agassiz	Lethbridge (Dryland)	Swift Current	Brandon	Melita	Morden	Winnipeg
Argentine:	I*	5.68	4.64	4.71	4.47	5.13	4.81	4.90
	T*	4.89	5.57	5.94	5.73	6.81	5.09	5.22
	R*	1.16	0.83	0.80	0.78	0.76	0.95	0.94
Swedish:	I	4.55	4.76	4.06	4.48	5.05	4.31	4.21
	T	5.08	5.47	5.10	5.89	6.52	4.88	5.06
	R	0.90	0.86	0.80	0.76	0.77	0.89	0.83
Golden:	I	3.86	4.11	4.60	4.70	4.25	5.12	3.67
	T	4.76	5.12	5.53	6.40	6.33	4.62	4.40
	R	0.81	0.80	0.83	0.74	0.67	1.11	0.83
Regina II:	I	5.05	5.29	3.95	4.08	5.00	4.63	4.48
	T	5.09	5.29	5.25	5.88	6.27	5.05	5.15
	R	1.00	1.00	0.76	0.70	0.80	0.92	0.87
Polish:	I	5.45	5.71	5.44	5.00	5.27	5.27	5.37
	T	1.47	1.81	1.48	1.33	1.78	1.62	1.37
	R	3.70	3.17	3.68	3.77	2.97	3.29	3.92
Arlo:	I	4.79	5.26	4.35	4.53	5.06	5.05	5.17
	T	1.25	1.40	1.24	1.27	1.60	1.25	1.32
	R	3.84	3.75	3.51	3.58	3.18	4.05	3.91
Gute:	I	4.91	5.26	4.41	4.33	4.93	4.81	5.29
	T	1.78	1.90	1.69	1.75	1.83	1.83	1.81
	R	2.77	2.78	2.61	2.49	2.70	2.64	2.92

* Isothiocyanate (I) and Thiooxazolidone (T) content expressed as mg. per gm. of fat-free meal

$$\text{Ratio(R)} = \frac{\text{Isothiocyanate}}{\text{Thiooxazolidone}}$$

TABLE 2.—ANALYSES OF VARIANCE OF ISOTHIOCYANATE AND THIOOXAZOLIDONE DATA

Source of variance	Degrees of freedom	Mean squares	
		Isothiocyanate	Thiooxazolidone
Variety	6	1.566**	60.62**
Station	6	0.591	1.844**
Interaction (Variety X Station)	36	0.265°°	0.360°°
Duplicates	49	0.039	0.033

** Significantly greater (at the 1% point) than variance due to interaction
°° Significantly greater (at the 1% point) than variance due to duplicates

TABLE 3.—VARIETAL MEANS FOR ISOTHIOCYANATE AND THIOOXAZOLIDONE FOR ALL STATIONS

Variety	Isothiocyanate	Thiooxazolidone
Polish-type		
Polish	5.36	1.55
Arlo	4.88	1.33
Gute	4.85	1.80
Argentine-type		
Argentine	4.91	5.60
Swedish	4.49	5.43
Golden	4.33	5.31
Regina II	4.64	5.42
95% fiducial limits	±0.39	±0.46

The sulphur-containing compounds were released enzymatically from the glycosides by employing an enzyme isolated from white mustard. The isothiocyanate content was determined by a modified argentimetric method (12) and the thiooxazolidone by a modification of Astood's procedure as reported by Wetter (13). The values are given in milligrams per gram of oil-free meal.

EXPERIMENTAL RESULTS

The isothiocyanate and thiooxazolidone contents of the samples were determined in duplicate and the means are reported in Table I. In addition the ratio of isothiocyanate content to thiooxazolidone content is shown.

Table 2 shows that the analysis of variance was set up so that the variance due to varieties, stations, interaction (variety x station), and duplicates was determined. Since the interaction variance was significantly greater than the variance due to duplicates, the interaction variance

TABLE 4.—STATION MEANS FOR ISOTHIOCYANATE AND THIOOXAZOLIDONE FOR ALL VARIETIES

Station	Isothiocyanate	Thiooxazolidone
Lethbridge	5.00	3.79
Melita	4.96	4.45
Agassiz	4.90	3.47
Morden	4.86	3.48
Winnipeg	4.73	3.47
Brandon	4.51	4.03
Swift Current	4.50	3.74
95% fiducial limits	±0.39	±0.46

was used to test the significance of the varietal and station differences. In addition, the significant interaction variance indicates that variation in duplicate analysis is not important in the final testing of varietal and station differences. The analyses of variance (Table 2) of the isothiocyanate content show that varietal means differ significantly but station means do not.

Since it has been shown that both components differ significantly in varieties and in some cases in stations it would be of interest to determine which are significantly different from one another. One method of doing this is to determine the 95 per cent fiducial limits for comparison of different means (10) i.e., for odds of 19 to 1 that a real difference exists between the means. The varietal and station means for the two sulphur components are given in Table 3 and 4 respectively along with 95 per cent fiducial limits.

From Table 3 one can see that real differences exist in the two factors being investigated. Polish has a significantly higher isothiocyanate content than either Swedish or Golden both of which are Argentine-types. The other varieties do not differ significantly from one another. The station means for isothiocyanate show no difference as is indicated in Table 4.

The thiooxazolidone content of varieties falls into two distinct and significantly different groups. The Polish-type is significantly lower in this material than is the Argentine-type; however, there is no significant variation within the types. The ratio of isothiocyanate to thiooxazolidone (Table I) demonstrates the same difference; that is, the ratio for the Argentine-type is much lower than for the Polish-type. In addition, it will be noted that there appears to be a difference within types as shown by comparison of this ratio for Gute with that for Polish and Arlo. Therefore, it appears that the ratio may be useful in distinguishing differences within types.

The means for the thiooxazolidone content for stations indicates that Melita is significantly higher than Agassiz, Morden, and Winnipeg. Since one is dealing here with stations in the extreme west and in the extreme east of the area, it is hazardous to attempt any interpretation of what this difference may mean. Further investigation may shed light on these differences between stations.

DISCUSSION

It will be noted from this investigation that the seven varieties used fall into two general groups, the Polish-types (Polish, Arlo, and Gute) and the Argentine-types (Argentine, Swedish, Golden and Regina II). The isothiocyanate content is similar for both types but the thiooxazolidone content is approximately three and a half times higher in the Argentine-type. In the present restricted test there are station differences but these cannot be adequately interpreted.

This preliminary investigation suggests that further valuable information might be expected if one were to assay the meals of various rapeseed varieties over a much wider area in Western Canada. In a study of this type one might be able to conclude whether the meals from rapeseed grown in more northern area of Western Canada differ significantly from those grown farther south and, if so, how and to what extent.

ACKNOWLEDGEMENT

The author wishes to thank J. W. White, of the Field Husbandry Department of the University of Saskatchewan, for making available the 49 samples that were used in this test. We also wish to thank B. Papish for his technical assistance in assaying the samples.

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A PORTABLE CHOPPER FOR FORAGE SAMPLES

A portable forage sample chopper has been used successfully for the last two years on the Illustration Stations of the Normandin, Quebec, Experimental Farm district.

An Atco power mower with a two cycle Atco-Villiers motor normally used for golf greens[1] was modified to make the new forage chopper. The original unit with a six-blade reel cut a swath 14 inches wide and the reel had a chain and gear drive through a friction clutch.

The grass box as well as the front and rear rollers were removed from the Atco unit and the handles were shortened. The drive chain to the rear rollers also was removed. The bed-knife was relocated at the front of the machine in order that material could be fed from the top. This left the blades easily accessible for sharpening.

A feeding trough was built over the fixed knife, the outer wall being approximately even with the cutting edge of the knife. Legs and skids were added and a collecting box fitted within them. Wheels could be added, if required. The trough and drawer were made of $\frac{1}{16}$ inch (18-gauge) sheet metal but 22-gauge would be satisfactory.

A partial separation of leaves and stems was noticed in the rectangular box first used and a 16-inch × 16-inch drawer, 8 inches deep, is now recommended. This will easily hold 4 pounds of green matter which can be chopped in little more than 1 minute. It takes 2 minutes to chop and weigh a 2-pound sample. The handful of forage should be spread against the wall of the trough and pushed in with an alternating up and down movement; without this movement too big a handful might jam the reel.

The vertical feed eliminates the need for feeder rolls and also makes the unit self-cleaning as the material passes readily through the chopper. Only the box needs brushing out between samples.

The total weight after modifications was 98 pounds and the over-all size was 32 inches high by 25 inches long and 18½ inches wide. The modifications were made by a local blacksmith at a cost of $60.00 for material and labour. The initial cost of the mower was $140.00.

Ease of assembly, quick chopping and light weight are the main characteristics of this machine; it is somewhat noisy.

ACKNOWLEDGEMENT

Acknowledgement is made to Lucien Genest, blacksmith of Normandin, Que., for mounting the machine.

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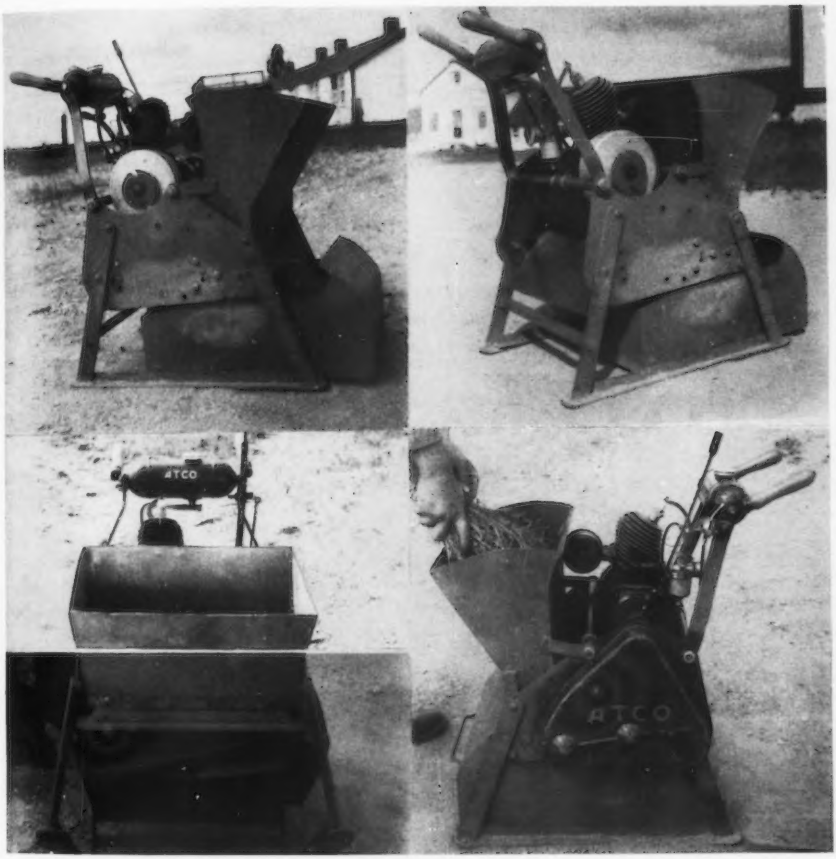
May, 15, 1959

[1] Obtained from Duke Lawn Equipment Ltd., Burlington, Ont.



Upper left: Side view showing the position of the fixed blade, the attachment of the feeding trough and the shape of the drawer. *Right:* Side view from behind; note the rear wall. *Lower left:* Front view—the trough has 10 inches at the opening and 2¼ inches at the bottom. Note the sliding bar fitting on the fixed blade at the bottom of the trough. *Right:* The chopper in action—Note the friction clutch.

NOTE ON BORON TOXICITY IN OATS

In a study of factors affecting persistence of Ladino clover, boron was applied broadcast at the rate of 35 pounds per acre of borax to a field of oats underseeded to a Ladino clover mixture. With the first noticeable oat growth, there appeared a definite chlorotic condition of the oat seedlings on plots receiving boron treatments, this condition showing as streaks within the plots. Seedlings on the affected portions of the boron-treated plots showed a distinctly yellow, and in some areas almost white, appearance as compared to the lush green tissue of the untreated plots.

Tissue analyses (2,3) for boron and nitrogen were made, and water-soluble boron determinations (1) of soil samples were carried out.

Analysis of chlorotic tissue, at 3 weeks after seeding, indicated 110 p.p.m. boron, while apparently healthy tissue revealed 6.1 p.p.m. boron at the same stage of growth. There was a rapid decline in the boron content of the oat tissue as the crop progressed to the heading stage. This was accompanied by a similar disappearance of apparent injury as shown by visual observations. The oat tissue from the boron-treated plots contained an average of 14.15 p.p.m. boron at heading stage as compared to 4.10 p.p.m. boron from untreated areas.

Nitrogen determinations of samples indicated no great difference in the nitrogen content of the distinctly yellow tissue and the green tissue. It was noted that the nitrogen content in the tissue samples decreased in a similar manner in tissue samples from all plots as the season progressed.

Soil analysis showed water-soluble boron to be present in the soil under the plants bearing yellow tissue to the extent of 1.41 p.p.m. The corresponding amount of boron in the soil under plants bearing green tissue growing within boron-treated plots was 0.84 p.p.m. boron. This suggests that the minimum toxic level of boron in the soil relative to oat tissue must be above 0.84 p.p.m. boron. On the average, the soils in the boron-treated plots contained 0.85 p.p.m. water-soluble boron. The plots not treated with boron had 0.36 p.p.m. water-soluble boron present.

Average yields from boron-treated and untreated plots were 61.6 and 61.4 bushels per acre respectively. These yields indicate that the boron toxicity noted earlier in the season had no harmful effect so far as yields were concerned, under conditions of this experiment.

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NOTE ON A PLOT-SIZE CANNING PEA VINER[1]

The handling of canning pea plots has always been difficult and often impossible due to the tedium of extracting the peas from the pods. Some types of machines pod peas effectively but only after the pods have been removed from the vines. Marion *et al.* (1) in 1951 developed a plot-size viner that would separate the peas from the vines and pods. This machine combined the basic principles of the large, commercial pea viner with the added feature that it was portable and could seemingly vine a large number of small samples of vines from experimental plots.

A vining machine was constructed according to the specific actions and plans of Marion (1). The only exception was the use of a 2-h.p. electric motor rather than the recommended $2\frac{1}{2}$-h.p. gasoline engine. This machine was tested to determine its usefulness in handling plots efficiently and rapidly.

The shortcomings of the machine were: 1) excessive vibration due to inadequate frame rigidity, 2) lack of sufficient power, 3) ineffective loading port latches, and 4) excessive plant debris in the shelled peas. Despite these apparent defects the principle was sound, and only modifications of the Marion viner are discussed in these notes. Figure 1 shows the original viner before any changes were made.

The primary object in changing the original viner was to increase its operating rate and efficiency so that it could handle a larger number of pea plots. Prior to modification the viner was capable of handling fewer than 50 5x10-foot plots a day. After modification 275 plots of the same size were handled (2).

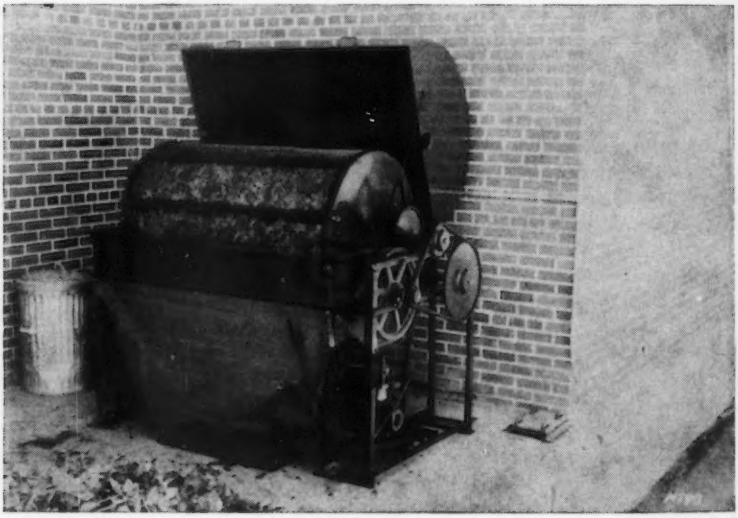

FIGURE 1. The viner as originally made, showing the light motor and unsupported framework and only a catch-all for collecting both shelled peas and plant debris.

[1] Contribution from the Horticulture and Soils Sections, Research Station, Lethbridge, Alta.

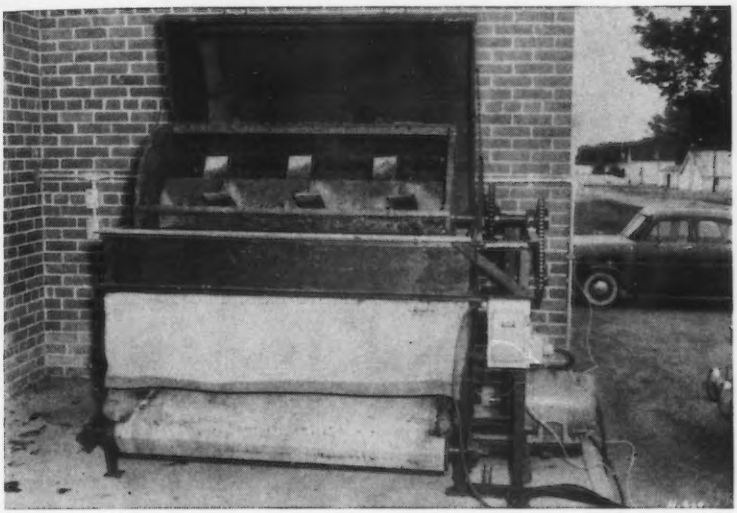

FIGURE 2. The improved viner, showing the rotary drop apron, reinforced frames and the heavier motor. The safety shield has been removed to show the drive mechanism.

The changes were as follows: 1) increasing the size of the structural members and utilizing extra bracing, 2) replacing the loading hatch thumbnut locks with quick-fastening snap locks for faster loading and unloading of the drum, 3) increasing the starting torque by using a 3-h.p., 3-phase electric motor, 4) replacing all high-friction bearings with self-aligning ball or roller bearings, 5) replacing the stationary drop-apron with a reverse-motion canvas apron for removal of plant debris, and 6) installing a $1\frac{1}{8}$-inch instead of $\frac{7}{8}$-inch beater shaft and reinforcing the beater paddle supports. The final, improved machine is shown in Figure 2.

The main modification to the viner was the installation of a canvas draper-type cleaning apron similar to that used on commercial viners. Driven from the second countershaft by means of a roller chain and sprocket drive, the apron could be stopped independently of the drum. Installed at an angle of 20 degrees to the horizontal, the apron was carried on 4-inch square rollers to agitate it in movement. Removable, wedge-shaped wooden slats were sewn into the apron to provide additional agitation. The apron carried the plant debris to the back of the machine and the peas rolled down the canvas and were collected at the front of the machine.

As originally planned by Marion (1), the viner was to have been a gasoline-powered, portable type. Experience after two seasons of operation has shown that it is better to bring the cut vines to the viner in order to utilize electrical power, which means smoother, more efficient operation of the viner. For optimum use of the machine a batch of approximately 30 pounds of pea vines (free from surface moisture) constitutes a full drum. The modified viner is hand-loaded through the loading hatch in the threshing drum. Reasonable care must be exercised when loading

TABLE 1.—EFFICIENCY OF IMPROVED PEA VINER BASED ON AVERAGE OF 50 PLOTS, EACH 5 FEET X 10 FEET

Variety	Vines	Peas shelled by		Shelling time		Viner losses of peas	Viner debris with peas
		Viner	Hand	By viner	By hand*		
	lb.	lb.	lb.	man-min.	man-min.	lb.	lb.
Alaska	13.46	0.89	0.68	11.37	40.35	0.04	0.1
Climax	25.35	1.37	1.73	11.85	45.63	0.05	0.3
Early Perfection	31.71	2.52	2.34	13.68	44.25	0.02	0.1

*Includes stripping pods from vines

to distribute the material evenly in the drum and to avoid overloading. After the peas are shelled, the beaten pulp is removed by hand or dumped on to the apron. Damp material caused by excessive dew or rainfall decreases viner efficiency and causes clogging and slippage of the drive belts.

The efficiency of the machine in terms of time and loss of peas was measured and these data are given in Table 1.

The efficiency of the viner was high and the vining operation was better than three times as rapid as hand-picking and -shelling of peas. Debris included very small, broken, and crushed peas. Bruising was light, less than 0.6 per cent by actual random sampling of several lots of 100 peas each. Varieties differed in their vining characteristics. Plots of heavy-vined varieties have to be smaller than those of lighter-vined types, e.g., Early Perfection versus Alaska. Slightly wilted vines were easier to shell and remove from the threshing drum.

ACKNOWLEDGEMENT

Acknowledgement is made to E. W. Thurston, Maintenance Supervisor at this Station, for assistance in modifying the viner.

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NOTE ON THE COMPARATIVE ACTIVITY OF MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS* SACC. FROM THE SAME SOURCE AS WHEAT PATHOGENS IN NATURAL AND STERILIZED SOIL

In order to replenish our supply of cultures of *Ophiobolus graminis* Sacc., a number of isolates were made from a collection of diseased Thatcher wheat plants sent to us in August 1957 from Cherry Point, Alberta. These specimens showed typical black basal stem lesions characteristic of the Take-all disease and bore numerous perithecia of *O. graminis* in their lower leaf sheaths. Several mycelial isolates were obtained from the stems following surface sterilization with silver nitrate and a number of monosporous isolates were secured from ascospores germinated in water agar. All of these isolates were subsequently grown on slants of potato dextrose agar in test-tubes.

Since a main purpose in making the isolates was to obtain some which would attack wheat seedlings severely in natural as well as in sterilized soil, a test was made in both soils during the winter of 1958. For this test inoculum of 16 isolates, 8 originating from single ascospores and 8 from mycelia, were grown on sterilized black soil containing 10 per cent corn meal in small Erlenmeyer flasks. After 3 to 4 weeks of growth the inoculum in each flask (50 gm.) was added at seed level to a 6-inch pot of either natural or steam sterilized soil. Red Bobs wheat sown on top of the inoculum (25 seeds per pot) was covered with about an inch of the same soil and the pots were watered and held in a dark chamber at 60° F. until the wheat seedlings had just emerged. They were then placed in a greenhouse compartment at higher temperatures and examined after disease symptoms were fully expressed.

The results of the 1958 test are summarized in Table 1. They show that in sterilized soil all of the monosporous isolates and the majority of the mycelial isolates caused severe infection of the wheat seedlings. The behaviour of the two types of isolates was in general not greatly different

TABLE 1.—COMPARATIVE SEVERITY OF INFECTION OF WHEAT BY MONOSPOROUS AND MYCELIAL ISOLATES OF *O. graminis* IN NATURAL AND STERILIZED SOIL

Isolate No.	Monosporous isolates		Isolate No.	Mycelial isolates	
	Natural soil	Sterilized soil		Natural soil	Sterilized soil
101	+	+	I	−	+
102	+	+	II	+	+
103	−	+	IV	−	+
104	+	+	V	−	+
105	+	+	VI	−	+
106	+	+	VII	−	−
107	+	+	VIII	−	+
108	+	+	IX	−	−

+ signs represent severe infection (over 75% of plants killed as seedlings).
− signs represent moderate to light infection (under 75% of plants killed as seedlings).

FIGURE 1. Relative effect of a monosporous isolate (108) and a mycelial isolate (V) of *O. graminis* on wheat: *left to right*, pots infested with 108 in sterilized soil, 108 in natural soil, V in sterilized, V in natural soil.

in such soil. In natural unsterilized soil, on the other hand, it was generally quite different. With one exception all of the monosporous isolates caused severe infection in natural soil whereas all but one of the mycelial isolates caused only moderate to light infection. If, as seems likely, the ratings observed in sterilized soil measure the relative pathogenicity of the isolates it would appear that highly pathogenic strains occur among both monosporous and mycelial isolates as has been previously noted by workers, e.g. Russell (3, 4) and Padwick (2), in other laboratories as well as in our own. The isolates reported on here, unlike those used by Russell and Patrick, were derived from the same source rather than from different sources. They might for this reason be expected to be more uniform and this seems to be true at least in respect to pathogenicity.

While the pathogenicity of the monosporous isolates is equal to if not greater than that of the mycelial isolates, this alone does not seem sufficient to account for their greater activity in natural soil. Possibly a factor other than or in addition to pathogenicity is operating in the natural soil. This may be an ability to compete more or less successfully with other soil organisms or to resist their products. It is probably the same as that recognized by Garrett (1) and termed by him "competitive saprophytic ability" in discussing the "competitive colonization of dead organic substrates". The monosporous isolates studied seemed to possess this quality more commonly than the mycelial isolates. However the occasional mycelial isolate, as for example Isolate II, appears to have this ability and the occasional monosporous isolate, for example Isolate 103, appears to lack it.

A second test similar to the one described but including more replicates (five instead of two) of fewer isolates, namely four of the monosporous and

four of the mycelial previously used, was made during the winter of 1959. It will be discussed in more detail in a subsequent paper but it is noteworthy that the results essentially confirm those of the first test. Figure I shows the relative behaviour of isolates 108 and V in the second test. It will be noted that the monosporous isolate was active in both natural and sterilized soil whereas the mycelial isolate though highly active in sterilized soil was relatively inactive in natural soil. In fact in natural soil infested with isolate V the wheat seedlings developed very much as they did in the check pots which had the culture medium minus the pathogen added to them.

It is suggested as a possible explanation of the results as a whole that different isolates or strains of *O. graminis* differ not only in pathogenicity but also in their ability to overcome the deleterious action on them of other soil organisms or their products. This latter attribute should be of particular value to a pathogen under natural conditions, and especially to one like *O. graminis* which functions mainly in or in association with the soil. In the material studied it was more commonly found in isolates from single ascospores than in those from mycelia but it was found present and lacking in both.

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